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(Continued)

NEOHETRAMINE: AN EXPERIMENTAL AND CLINICAL EVALUATION IN ALLERGIC STATES*†‡

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THERE has been a great deal of interest shown in the past few years in the ability of certain chemicals to antagonize histamine. A number of these chemicals have been of use clinically in the handling of certain allergic states. It is the purpose of this paper to present certain experimental and clinical results concerning a new antihistaminic agent called Neohetramine (2-(N-dimethyl-aminoethyl-N-p-methoxybenzyl)-amino-pyrimidine monohydrochloride).

TOXICITY

This substance is very soluble and is quite stable in an aqueous solution. Its toxicity is lower than any of the other antihistaminics. The oral L.D. 75 for guinea pigs is 1,100 mg. per kilogram. Two per cent Neohetramine in neutral isotonic solution can be injected intradermally without any noticeable change.

Seventeen patients were hospitalized. Each of these patients had a urinalysis, complete blood count, and an electrocardiogram. They were given 200 to 400 mg. of Neohetramine daily in divided doses for four days. On the last day of medication, the urinalysis, complete blood count, and electrocardiogram were repeated. These showed no significant changes. There was also no noticeable change in the blood pressure of these patients during and after medication.

Eight patients were hospitalized and electroencephalograms were taken on each. Then two patients received 200 mg. daily of Benadryl, two patients received 200 mg. daily of Pyribenzamine, two received 200 mg. daily of Thephorin, and two received 200 mg. daily of Neohetramine. On the second day the electroencephalograms were repeated. It was noted with all that there

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was a sporadic increased fast activity superimposed on normal alpha rhythm. These did not appear to be artefacts because they appeared in all tracings while the patients were on the drug. There was an occasional lowering of the amplitude of the waves while the medication was being administered.

PROTECTIVE EFFECT OF NEOHETRAMINE AGAINST HISTAMINE IN VIVO

Three milligrams per kilogram of body weight of Neohetramine were injected intraperitoneally into guinea pigs. Fifteen minutes later the histamine base was injected into the dorsal vein of the penis. The results obtained are recorded in Table I. Thus using 3 mg. per kilogram of body weight it was

TABLE I. EFFECT OF INJECTING VARYING DOSES OF HISTAMINE INTRAVENOUSLY INTO GUINEA PIGS FIFTEEN MINUTES AFTER INJECTING 3 MG. PER KILOGRAM OF NEOHETRAMINE INTRAPERITONEALLY

DOSE OF HISTAMINE BASE INJECTED INTO DORSAL VEIN (MG./KG.)	NUMBER OF GUINEA PIGS THAT DIED	PER CENT MORTALITY
0.4	1 of 6	16
0.5	2 of 8	25
0.8	3 of 5	60
1.0	6 of 12	50
2.0	4 of 6	69
2.5	7 of 7	100

necessary to use 2.5 mg. per kilogram of histamine base to obtain 100 per cent fatalities. We had determined that the lethal dose of histamine base fatal to 100 per cent of the guinea pigs when injected into the dorsal vein of the penis was 0.4 mg. per kilogram. Therefore, Neohetramine protects against six times the lethal dose. The comparison with the 100 per cent lethal dose necessary when other drugs are used in the same circumstances was favorable: It is 2.4 mg. per kilogram of histamine base for Antergan, 50 mg. per kilogram for Neoantergan, 2 mg. per kilogram for Benadryl, 15 mg. per kilogram for Pyribenzamine, and 4.5 mg. per kilogram for Thephorin.[1]

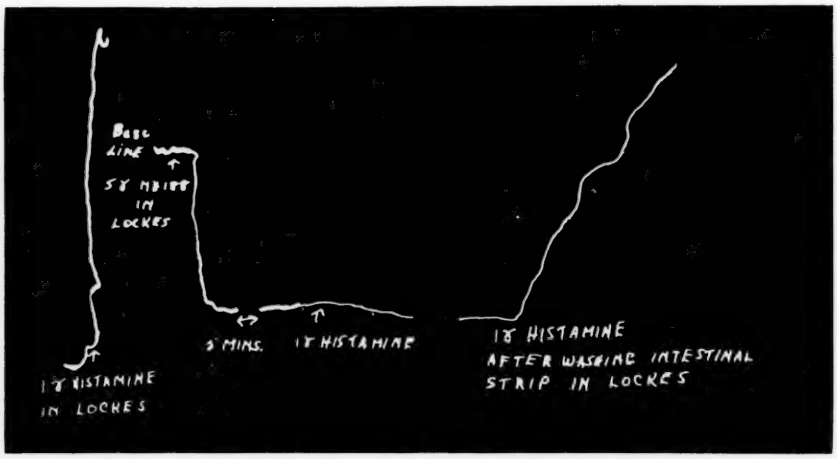

Fig. 1.—Neohetramine protection versus histamine in vitro.

PROTECTIVE EFFECT OF NEOHETRAMINE AGAINST HISTAMINE IN VITRO

Studies were carried out using the Schultz-Dale bath with guinea pig intestinal strips. One gamma per cubic centimeter of histamine base gave an excellent contraction of over one inch. It was found that this contraction was completely inhibited by adding concentration of 5 gamma per cubic centimeter of Neohetramine to the bath. It had been determined that 1 gamma of Pyribenzamine, 2 gamma of Thephorin, 2.5 gamma of Antergan, 1 gamma of Neoantergan, and 5 gamma of Benadryl are each capable of the same inhibition.[2]

PROTECTIVE EFFECT OF NEOHETRAMINE AGAINST ANAPHYLAXIS IN VIVO

Guinea pigs were actively sensitized with horse serum. It was possible to produce fatal anaphylactic shock in five of five animals by injecting 1 c.c. of horse serum into the dorsal vein of the penis of the animal. Varying protective doses of Neohetramine were injected intraperitoneally fifteen minutes prior to the injection of the shocking dose of horse serum. It was found that in our series 5 mg. per kilogram of Neohetramine were capable of 100 per cent protection. (Table II.) Others[2] have done similar experiments using Pyribenzamine and Benadryl as the protective agents. They, however, used a smaller amount of antigen. The results are comparable with those obtained here. With 3 mg. of Pyribenzamine, using ten animals, they obtained 100 per cent survival, and with 3 mg. of Benadryl, using ten animals, they obtained 100 per cent survival. It is admitted that a small series of animals was used in this particular study and that this phase of the investigation will bear corroboration.

TABLE II. PROTECTIVE EFFECT OF VARYING DOSES OF NEOHETRAMINE IN ACTIVE ANAPHYLAXIS

DOSE OF NEOHETRAMINE (MG./KG.)	NUMBER OF GUINEA PIGS THAT DIED	PER CENT MORTALITY	PER CENT SURVIVAL
2	3 of 4	75	25
3	1 of 4	25	75
4	1 of 4	25	75
5	0 of 3	0	100

PROTECTIVE EFFECT OF NEOHETRAMINE AGAINST ANAPHYLAXIS IN VITRO

Studies were carried out on the intestinal strip with the Schultz-Dale bath using guinea pigs which had been adequately sensitized with horse serum. The intestinal strips showed a very marked contraction with 0.5 c.c. of undiluted horse serum. We were unable to inhibit the contraction caused by the horse serum when using a concentration of 10 gamma per cubic centimeter of Neohetramine.

THE EFFECT OF NEOHETRAMINE TAKEN ORALLY ON HISTAMINE AND ON ANTIGEN-ANTIBODY WHEALING

Ragweed-sensitive patients were skin tested with ragweed and also with histamine, 1:10,000 dilution. The wheals which formed within ten minutes were traced and recorded on transparent paper. The patients were then given 200 mg. of Pyribenzamine orally and in one hour intradermal tests were repeated. It was found that the wheals and flares were decreased in size. This

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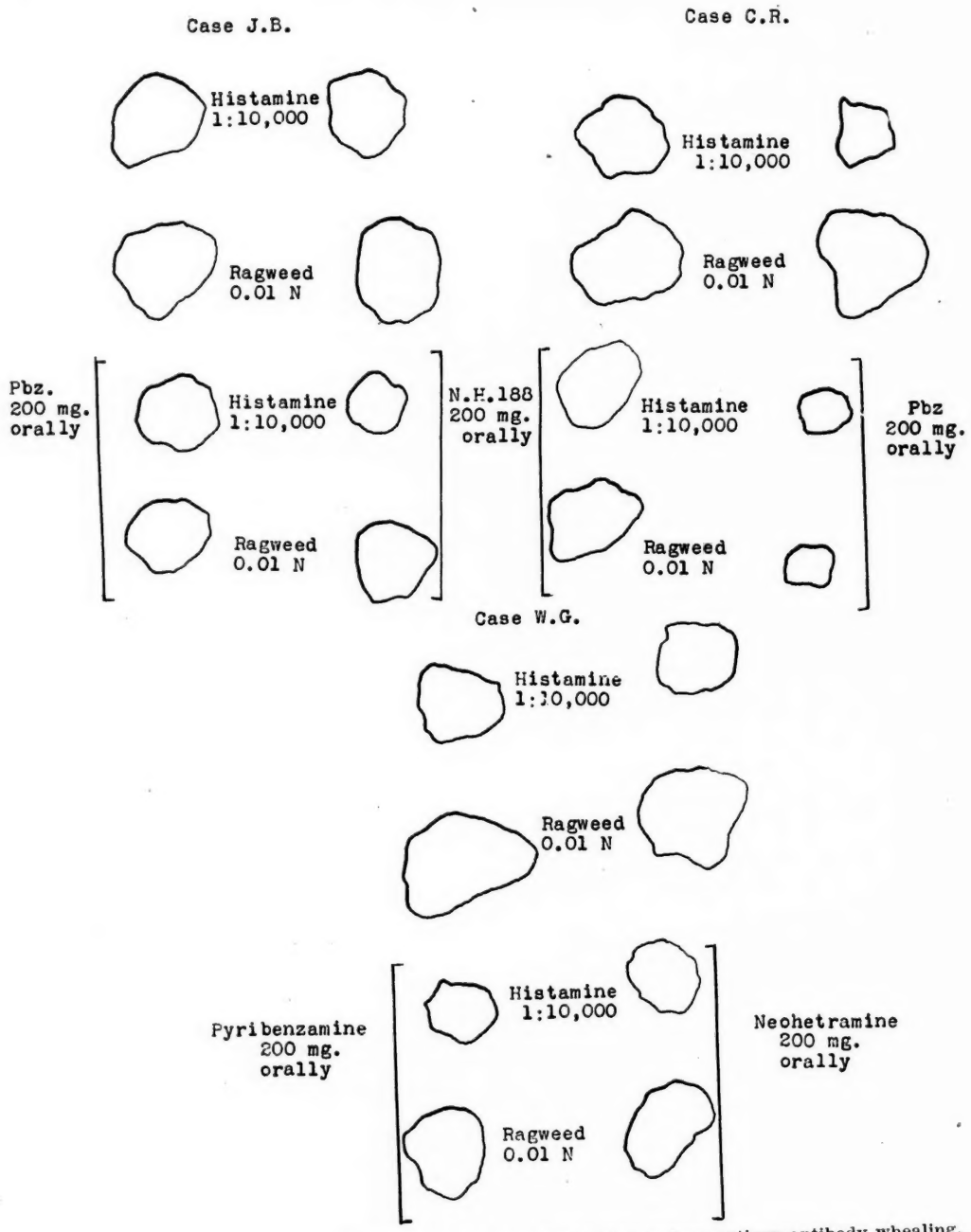

Fig. 2.—The effect of Neohetramine taken orally on histamine and on antigen-antibody whealing.

same procedure was repeated on another day using the same patients. The antihistaminic drug used this time was Neohetramine in a dose of 200 milligrams. Here, also, the wheals and flares were decreased in size. The inhibition in both cases was of small amount. There appeared to be slightly less inhibition

with Neohetramine (Fig. 2), although it should be stated that the effect of orally administered drugs on the skin test from histamine or ragweed need not necessarily be quantitative.

THE EFFECT OF LOCAL INJECTION OF NEOHETRAMINE ON HISTAMINE AND ANTIGEN-ANTIBODY WHEALING

Ragweed-sensitive patients were skin tested with ragweed antigen and with histamine, 1:10,000 dilution, injected intradermally. The wheals that were formed were traced and recorded on transparent paper. Concentrations of the antigen and Neohetramine were mixed in order that the same amount of antigen was injected intradermally as before and there was thus a concentration of 0.25 per cent of Neohetramine in the mixture. The same procedure was used with histamine and Neohetramine, so that the mixture contained a final concentration of 1:10,000 of histamine and 0.25 per cent of Neohetramine. It was found that the wheals were smaller when the antigen or the histamine was mixed with Neohetramine before injection (Fig. 3).

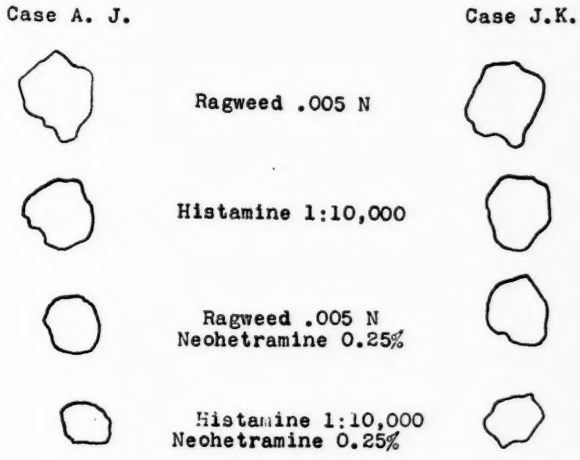

Fig. 3.—The effect of local injection of Neohetramine on histamine and antigen-antibody whealing.

SPIROMETRIC EVALUATION OF NEOHETRAMINE IN ASTHMA

Twenty-one cases of different types of bronchial asthma were studied using Neohetramine. All of the patients had active asthmatic symptoms and/or râles, and rhonchi were heard on examination of their chests. Their vital capacity was determined immediately prior to the evaluation of the drug by use of the McKesson-Scott apparatus. This was done three times in all cases and the average was the figure recorded. A triturated tablet of 50 mg. of Neohetramine was then administered orally at once. The readings were taken of the vital capacity in the same manner one-half hour and one hour later. The dose of 50 mg. was chosen to relate as closely as possible to therapeutic trials of the drug. It can be noted in Table III that six patients had an increased vital

capacity in one-half hour. Fifteen did not improve or their capacity decreased. In one hour, fourteen improved while five did not. One patient improved in one-half hour with clinical improvement, and in one hour the vital capacity decreased and the patient's clinical improvement decreased. Of those who improved with the drug, seven had an improvement of approximately 25

TABLE III. SPIROMETRIC RESULTS FOLLOWING NEOHETRAMINE

PATIENT	DIAGNOSIS	VITAL CAPACITY IN LITERS		
		0	½ HR.	1 HR.
A. H.	Chronic asthmatic bronchitis	1.7	2.0	2.1 (Clinical improvement)
J. C.	Chronic asthmatic bronchitis	2.7	2.7	2.4 (Slight improvement)
W. A.	Chronic asthma bronchitis	2.5	3.2 (Clinically better)	2.7 (Wheezing again)
J. V.	Chronic asthmatic (ext. and int.)	3.0	2.6	3.4
D. P.	Bronchial asthma (ext.)	2.6	2.3	2.1
C. M.	Bronchial asthma (ext. and int.)	3.2	3.2	3.4
S. R.	Bronchial asthma (ext. and int.)	3.4	3.2	3.6 (Slight improvement)
C. B.	Bronchial asthma (ext. and int.)	4.0	4.0	4.8 (Slight improvement)
J. C.	Bronchial asthma (ext. and int.)	2.1	3.0	2.9 (Clinically better)
H. H.	Bronchial asthma (int.)	2.1	1.9	2.1
C. M.	Bronchial asthma (ext.)	2.8	2.8	2.9
A. P.	Bronchial asthma (ext.)	4.6	4.7	4.7
W. S.	Bronchial asthma (ext.)	3.1	3.0	3.0
T. R.	Bronchial asthma (ext.)	3.2	3.1	3.3
D. J.	Bronchial asthma (ext. and int.)	1.3	1.2	1.3
J. R.	Bronchial asthma (ext.)	5.2	5.2	5.5
W. E.	Bronchial asthma (ext.)	2.4	3.7	4.3 (Clinical improvement)
H. F.	Chronic asthmatic bronchitis	2.0	2.1	2.6 (Clinical improvement)
W. B.	Bronchial asthma (int.)	4.2	4.1	4.0
E. W.	Bronchial asthma (ext. and int.)	3.4	3.4	3.2
J. C.	Chronic asthmatic bronchitis	2.4	2.1	3.1

Ext., Extrinsic type.
Int., Intrinsic type.

per cent or more of their original vital capacity. Nine had clinical improvement, and of these seven had an increase of 25 per cent or more of their vital capacity. One had an increase of 79 per cent of the original capacity. One patient who improved clinically had a decrease of his capacity, and one had only a slight increase. During the test no definite correlation could be made as to the type of asthma and the increase or decrease in vital capacity. However,

many of those who did have an increase were those in whom extrinsic asthma was present. It is obvious that these findings are not clinically significant. It certainly cannot be concluded from the foregoing that the drug is therapeutically useful in bronchial asthma.

CLINICAL EVALUATION OF NEOHETRAMINE

Neohetramine is available in 50 mg. tablets which are scored. The dosage used was 50 mg. every four hours as needed. A total of 249 patients received the drug in order to evaluate its efficacy. Good relief was obtained in many. The relief, when obtained, from the drug from one tablet, lasted, as with the other drugs, approximately three to six hours. The great value of this drug was the very low percentage of side reactions. The results were divided into No Relief, Slight Relief, Moderate Relief, and Complete Relief (Table IV). Some placebo controls were used. In many instances the drug was readministered for several times after it was discontinued in order to observe its repeated effect.

TABLE IV. CLINICAL EFFECT OF NEOHETRAMINE

NUMBER	DIAGNOSIS	NONE (%)	SLIGHT (%)	MODERATE (%)	COMPLETE RELIEF (%)
124	Hay fever	18	22	27	33
41	Allergic rhinitis	20	27	24	27
33	Bronchial asthma	37	24	27	12
11	Atopic dermatitis	18	73	9	0
20	Urticaria	25	25	20	30
6	Migraine	17	33	0	50
8	Contact dermatitis	0	38	50	12

Hay Fever.—One hundred and twenty-four patients with seasonal hay fever received Neohetramine. Eighteen per cent reported no relief; 22 per cent, slight relief; 27 per cent, moderate relief; and 33 per cent, complete relief. Because many patients received concomitant injection therapy, benefit was recorded by comparison with periods when the drug was not taken.

Perennial Allergic Rhinitis.—Forty-one patients with perennial allergic rhinitis received Neohetramine therapy. Twenty per cent obtained no relief, 27 per cent had slight relief, 24 per cent had moderate relief, and 29 per cent had complete relief.

Bronchial Asthma.—Thirty-three patients with bronchial asthma of all types received Neohetramine. As with the other antihistaminic drugs, only a low percentage obtained some relief, and the majority of those who did were in the extrinsic asthma group. Thirty-seven per cent obtained no relief; 24 per cent, only slight relief; 27 per cent, moderate relief; and 12 per cent, complete relief.

Atopic Dermatitis.—The results in a total of eleven patients with atopic dermatitis who had received Neohetramine were as follows: Eighteen per cent had no relief, 73 per cent had only slight relief of the pruritus, and 9 per cent had moderate relief. This low figure is comparable with that obtained with the other antihistaminic drugs.

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Contact Dermatitis.—Eight patients with contact dermatitis took Neohetramine. Thirty-eight per cent received slight relief from the pruritus; 50 per cent, moderate relief; and 12 per cent, complete relief.

Urticaria and Angioneurotic Edema.—Twenty patients with urticaria and angioneurotic edema were treated with Neohetramine. Good results were obtained: 30 per cent had complete relief; 20 per cent, moderate relief; and 25 per cent, slight relief; 25 per cent received no relief at all.

Migraine.—Only six patients with migraine headache received Neohetramine. Fifty per cent obtained complete relief, while 33 per cent had slight relief and 17 per cent no relief at all.

Under a miscellaneous group one patient with Schoenlein's purpura received only slight relief with Neohetramine; one with pruritus ani received no relief; two patients with serum disease-drug allergy received moderate and marked relief; one patient with an urticarial reaction to injection therapy received marked relief, and, finally, one with acne rosacea received no relief.

Side Reactions.—Of the 249 patients who used Neohetramine in our series, only 10 per cent had any side reactions. This percentage is certainly much lower than that noted for most antihistaminic drugs. Twenty-four per cent of those who complained of reactions had nervousness and palpitation, 16 per cent complained of nausea and vomiting, and 16 per cent complained of insomnia. Drowsiness was not a major factor, and only 16 per cent of those having reactions complained of it. Eight per cent of those who had side effects complained of dizziness, 8 per cent complained of constipation, eight per cent complained of diarrhea, and, finally, 4 per cent had headache from the drug (Table V).

TABLE V. NEOHETRAMINE SIDE EFFECTS (10 PER CENT OF TOTAL)

SIDE REACTION	PERCENTAGE OF TOTAL REACTIONS
Nervousness	24
Nausea	16
Insomnia	16
Drowsiness	16
Dizziness	8
Diarrhea	8
Constipation	8
Headache	4

CONCLUSIONS

Neohetramine was found to be as effective as the other antihistaminic drugs experimentally and clinically. We have attempted to follow as closely as possible the methods of workers who have used the other drugs. This was done in order to form some comparison for evaluation of this drug.

Toxicity studies were carried out and it was found that there was no appreciable change in a complete blood count, urinalysis, blood pressure, or electrocardiogram of patients taking the drug. Electroencephalogram studies showed some sporadic fast activity while the drug was being taken. Several untreated hay fever patients showed no significant changes in blood count and urine examinations after having taken the drug over a period of four to six weeks.

In its antihistaminic activity in vivo, Neohetramine was found to be slightly better than Benadryl and about the same as Antergan. In vitro, its histamine protective value was the same as Benadryl but not as great as Antergan. Enough evidence has been obtained to say that this drug has good antianaphylactic properties in vivo but little protective properties in vitro.

It has been shown also that Neohetramine is capable locally of having an inhibiting action on histamine and on antigen-antibody whealing formation. When orally administered the inhibition is less.

On spirometric studies there was not much appreciable change of the vital capacity. With seven of twenty-one patients we have been able to demonstrate an increase of over 25 per cent of the original capacity. The increases were noticed mostly in those in whom extrinsic asthma was present. This compares with Benadryl.[3] It follows that the drug may be of slight value in extrinsic asthma, as are the other antihistaminic drugs.

Clinically, Neohetramine is valuable in the symptomatic treatment of seasonal hay fever. Only 18 per cent of the patients had no relief. This is the same percentage that Feinberg[1] obtained with Pyribenzamine. Of the patients with perennial allergic rhinitis, only 20 per cent had no benefit from the drug. Of those with asthma, 37 per cent had no relief, but a total of 39 per cent were benefited. This group included mostly those with extrinsic asthma. This compares with 28 per cent relief obtained with Pyribenzamine. Of those with atopic dermatitis, 73 per cent had slight relief of the pruritus and only 9 per cent had moderate relief. Only 25 per cent had no relief from the drug in urticaria and angioneurotic edema. This also compares favorably with Pyribenzamine. Thirty per cent had complete relief and 20 per cent had moderate relief. Of the patients with contact dermatitis, 62 per cent had good relief from the pruritus, and 38 per cent slight relief. Patients complaining of migraine headache who took the drug had good results; 50 per cent obtained complete relief. However, the series is too small to draw accurate conclusions in this type of headache.

The percentage of side reactions with Neohetramine is extremely low. In our series it was only 10 per cent of the patients who had taken the drug. With Pyribenzamine[1] reactions were reported in 23 per cent of those who had the drug; with Benadryl, 50 per cent. Nervousness and palpitation or a benzedrine-like effect was the greatest complaint. Only a small percentage had drowsiness and dizziness as compared with the effects of the other drugs.

SUMMARY

1. Neohetramine, a new antihistaminic drug, has been found to have good antihistaminic and good antianaphylactic (in vivo) properties.

2. Toxicity studies were carried out on seventeen patients in whom blood counts, urinalyses, blood pressure readings, electrocardiograms, and some electroencephalograms were done.

3. Whealing studies were done which portrayed the inhibiting action of the drug.

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4. Spirometric studies were carried out on twenty-one patients with bronchial asthma.

5. Clinically Neohetramine was found to be of as much value as the other antihistaminic drugs in the treatment of allergic states.

We are grateful for the technical assistance of M. I. Levine, M.D., and M. Kalser, M.D.

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CANTHARIDIN BLISTER STUDIES*†‡

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INTRODUCTION

THE intraepithelial blister which develops as the result of the application of cantharides to the intact skin has been used extensively for the investigation of inflammatory changes and immunoreactions. Studies such as were originated by Neumann[1] in 1878 included work on capillary permeability (Müller,[2] Gänsslen,[3] Petersen,[4] Cornbleet and Klein[5]), on the chemistry of the inflammatory exudate (Eppinger, Kaunitz, and Popper[6]), on the recovery of pathogens from the blister fluid (Benedek[7]), and on other biologic phenomena; for example, the von Pirquet test (Buschke and Kuttner[8]). "Cellular" antibodies (Urbach[9]) as well as circulating antibodies (Spain and Newell,[10] Parlato[11]) have been successfully transferred from blister fluid to the skin of normal individuals.

The cellular content of the cantharidin blister was studied by Tommasini[12] in 1904; Kauffmann[13] examined the differential count of blisters produced in the same patient in regular intervals throughout the course of various diseases. He established the fact that changes in its noneosinophilic components closely reflect changes in the "reactivity" of the organism as a whole. The changes in the blister count, however, do not parallel those found in the blood (Sonnenfeld and Leffkowitz[14]) and are influenced by physiologic incidents as menstrual cycle (Hossmann[15]) and pregnancy.

Few data have been collected on the percentage of eosinophils in cantharidin blister fluid (Tommasini,[12] Goodman[16]). Kauffmann regarded the presence of from 0 to 3.25 per cent as their normal range. He concluded on the basis of his experiments that while the character of the noneosinophilic cells is determined by nonspecific regulations, "much more specific factors" seem to account for the eosinophilic response.

We have attempted to investigate in this study a number of those factors which in accordance with theoretical concepts might cause the occurrence of eosinophilia in cantharidin blister fluid. For this purpose we have (1) reexamined the percentage of eosinophiles found in cantharidin blisters produced on the skin of healthy persons and patients with various diseases and (2) tried to call forth their appearance in those in whom none had been previously present.

PART I. VARIATION IN PERCENTAGE OF EOSINOPHILES FOUND IN CANTHARIDIN BLISTER FLUID OF PATIENTS WITH VARIOUS DISEASES AND OF NORMAL CONTROLS

Experimental Procedure.—The procedure used by different investigators for the production of cantharidin blisters has only slight modifications. One centimeter squares of emplastrum cantharidis N. F. VII were applied to the flexor surface of the upper arms, two on each side. They were covered with

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gauze and held in place with adhesive tape. Sixteen hours later tape, gauze, and the plaster were removed, fluid withdrawn with syringe and needle, and separate smears made of the contents of each of the four blisters.

Application of cantharidin and withdrawal of fluid are painless. Slight itching or burning during the early hours of blister formation causes occasional insignificant discomfort. The blister site heals rapidly; in most cases a slight transitory pigmentation can be observed. Lesions which remained uncovered seemed to restore epithelium in a shorter period of time than those covered by protective bandages. No secondary infection was seen.

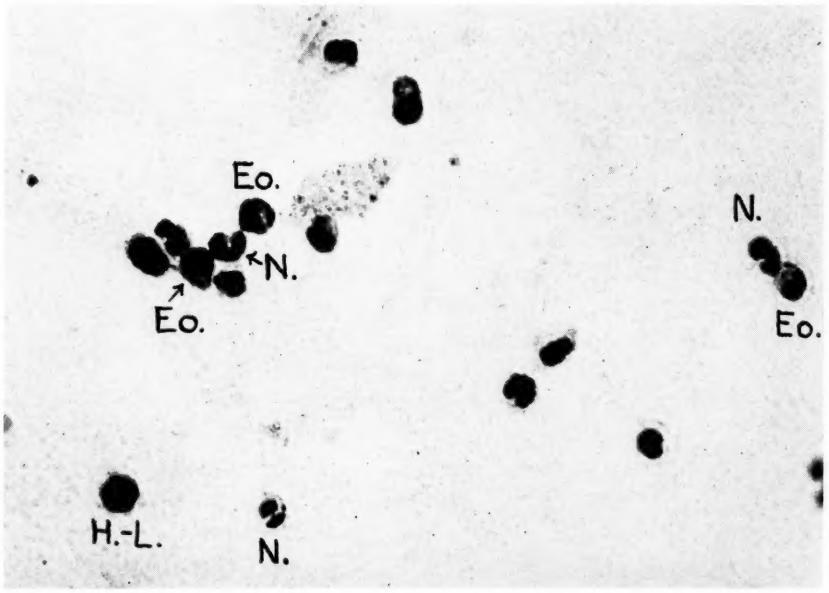


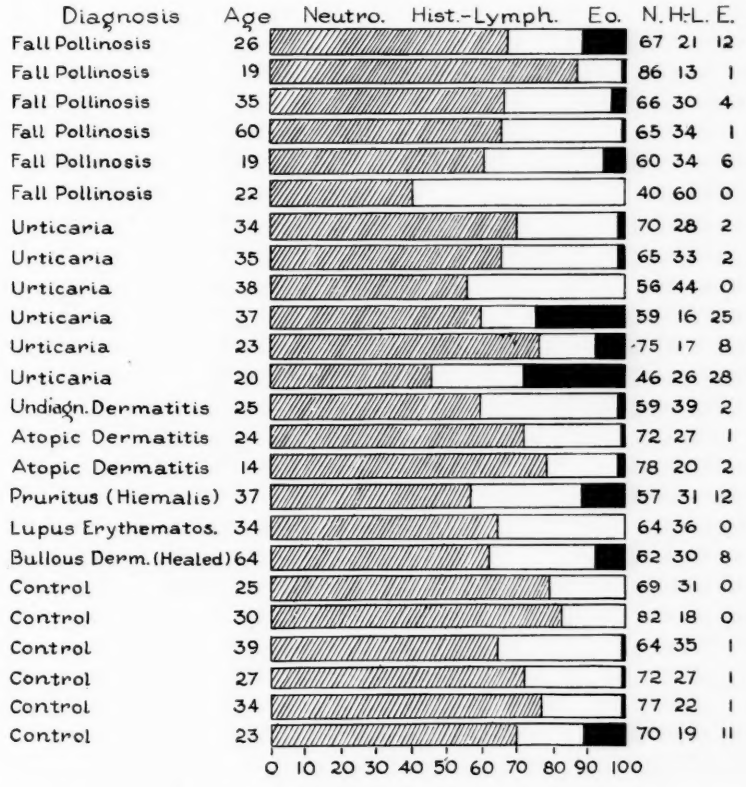
Fig. 1—Smear of cantharidin blister fluid sixteen hours after application of emplastrum cantharidis. In the specimen, eosinophiles are easily recognized by the color of their granules in contrast to light blue background. Eo., Eosinophiles; N., neutrophiles; H.-L., lymphohistiocytes.

The blisters varied considerably in size; constitutional factors seem to stimulate or retard blister formation (Buday and Feledi[17]). Only one of our patients failed to develop blisters.

The interval between application and withdrawal, sixteen hours, was selected because it afforded a satisfactory number of cells in the blister fluid; at the same time the exudate was not yet thick enough to require centrifugation and resuspension of the cellular material.

The staining technique recommended by Kauffmann[13] was found to be adequate. It was used exclusively throughout this study. This consisted of fixation with methyl alcohol for three minutes; staining with a mixture of equal parts of May-Grünwald stain and distilled water for five minutes; counterstaining with methylene blue, 1 per cent aqueous solution for ten seconds; rinsing with distilled water. Rapid drying: Kauffmann suggests the use of filter paper, but we have found little difference between paper-dried and air-dried preparations.

Two hundred cells were counted in each smear. The identification of cells in stained specimens is not difficult. The cells are divided in three well-defined groups. The first, polymorphonuclear neutrophilic leucocytes, "neutrophiles," represent with few exceptions the major portion of the cellular material. The second, designated by Kauffmann[13] as "lympho-histiocytes," are nongranulated basophilic cells with round, oval, or bean-shaped nuclei. A number of these cells were shown to be phagocytic; Kauffmann concludes, therefore, that this group consists of lymphocytes as well as of cells derived from the reticulo-endothelial system or, more specifically, from the adventitia of the blood vessels which are involved in the inflammatory process. The third, eosinophiles, are prominent by the color and brilliance of their granules (Fig. 1).


Diagnosis
Age
Neutro.
Hist.-Lymph.
Eo.
N. H-L. E.
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Fall Pollinosis 19 86 13 1
Fall Pollinosis 35 66 30 4
Fall Pollinosis 60 65 34 1
Fall Pollinosis 19 60 34 6
Fall Pollinosis 22 40 60 0
Urticaria 34 70 28 2
Urticaria 35 65 33 2
Urticaria 38 56 44 0
Urticaria 37 59 16 25
Urticaria 23 75 17 8
Urticaria 20 46 26 28
Undiagn. Dermatitis 25 59 39 2
Atopic Dermatitis 24 72 27 1
Atopic Dermatitis 14 78 20 2
Pruritus (Hiemalis) 37 57 31 12
Lupus Erythematos. 34 64 36 0
Bullous Derm. (Healed) 64 62 30 8
Control 25 69 31 0
Control 30 82 18 0
Control 39 64 35 1
Control 27 72 27 1
Control 34 77 22 1
Control 23 70 19 11
0 10 20 30 40 50 60 70 80 90 100

Fig. 2.—Distribution of cells in the differential counts of cantharidin blisters raised on twenty-four patients with various diseases and normal controls.

The blister fluid from each of the four sites of cantharidin application showed occasional slight fluctuations of the number of neutrophiles and lympho-histiocytes, respectively, but the number of eosinophiles with which this study is primarily concerned appeared to be fixed within a narrow range. The percentage of eosinophiles listed in Table I and the differential blister counts shown in Fig. 2 represent the average obtained in accordance with our experimental procedure from the four individual blisters on each person. In each case, a differential blood count was made at the time of the withdrawal of blister fluid.

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TABLE I. AVERAGE PERCENTAGE OF EOSINOPHILES IN PERIPHERAL BLOOD AND BLISTER FLUID OF PATIENTS WITH VARIOUS DISEASES AND NORMAL CONTROLS

DIAGNOSIS	NUMBER OF CASES	AVERAGE PERCENTAGE OF EOSINOPHILES IN THE PERIPHERAL BLOOD	AVERAGE PERCENTAGE OF EOSINOPHILES IN THE BLISTER FLUID
Fall pollinosis, untreated	12	3.7	3.6
Bronchial asthma	10	4.8	2.8
Urticaria	12	2.9	10.6
Skin disorders other than urticaria	15	7.3	6.6
Endocrine disorders including diabetes mellitus	10	0.8	1.9
Cardiovascular disease	14	2.9	2.2
Gastrointestinal disease	10	3.4	5.2
Controls	15	1.5	1.9

Ninety-eight subjects participated in the experiment. Patients belonged to the following categories: fall pollinosis, 12*; chronic bronchial asthma, 10; urticaria, 12; skin disorders other than urticaria, 15; endocrine disorders including diabetes mellitus, 10; cardiovascular, 14; gastrointestinal disorders, 10. Controls consisted of medical students and of staff members. Fourteen persons agreed to a second application of cantharidin, five to fourteen days after the completion of the first series of blisters.

Results.—The distribution of cells in differential blister counts of eighteen patients with various diseases and six healthy controls is shown in Fig. 2. The range of neutrophiles extends from 40 to 86 per cent; of lymphohistiocytes, from 13 to 60 per cent; of eosinophiles, from 0 to 28 per cent. The average percentage of neutrophiles in this group is 65.9; of lymphohistiocytes, 28.8; of eosinophiles, 5.3. The number of lymphohistiocytes counted in our specimens is considerably higher than the average percentage found by Kauffmann; this might be due to differences in the preparation of the cantharidin plaster used in the respective studies, a possibility which Kauffmann himself has repeatedly emphasized. Table I lists the average percentage of eosinophiles in blood and blister fluid of the ninety-eight individuals studied. In the different categories the average number of eosinophiles varied from 0.8 to 7.3 per cent in the blood and from 1.9 to 10.6 per cent in the blister fluid. The highest number of eosinophiles was found in the blister fluid of patients with urticaria. Our findings confirmed the experience of previous workers, that no correlation exists between eosinophilia in blood and blister fluid; yet in some of the groups, for example, skin disorders other than urticaria, the similarity of the figures is impressive.

Twenty-four of ninety-eight patients had 1 per cent or less eosinophiles in the blister fluid. It had been one of the objectives, if not the major objective, of the first part of this study to single out a group of individuals whose blisters were free from eosinophiles. Such a group was expected to permit comparative studies of the effect on the percentage of blister eosinophiles of experimental factors suspected to produce eosinophilia. It was the same consideration which caused us to eliminate patients with infectious diseases from our study, since

*Untreated; blisters obtained in July, 1947.

Kauffmann[13] had demonstrated the lability of the differential cell count of cantharidin blister fluid during the course of conditions as pneumonia, polyarthritis, and tuberculosis.

The differential blister counts found after repeated cantharidin application were so similar to those obtained in the original blisters that a separate listing did not seem indicated.

Discussion.—The interpretation of the findings which are summarized in Table I is limited by the small number of patients in each category. Furthermore, none of the categories was sufficiently uniform in itself. The group of patients with skin disorders other than urticaria, for instance, consisted of 3 with atopic dermatitis, 3 with undiagnosed dermatitis, 4 with dermatitis venenata, 2 with pruritus, 1 with bullous dermatitis, 1 with lupus erythematosus, and 1 with pityriasis rosea. It is interesting to note that the percentage of eosinophiles which was present in the blister fluid in patients with various skin diseases compares well with the degree of tissue eosinophilia which Burkhart and Montgomery[18] demonstrated in the same disorders by histologic methods. The variations which seem to exist deserve further investigation. In the urticarial group, for instance, the highest percentage of eosinophiles was found in the blister fluid of patients whose urticaria had been present for not more than three months; in the group of patients with gastrointestinal disorders those with regional ileitis showed an increase in eosinophiles of the blister fluid which accounts for the average of 5.2 per cent. The study is being continued with a large group of patients to establish the significance of these findings.

PART IIA. EOSINOPHILE BLISTER COUNT IN NORMAL AND ALLERGIC INDIVIDUALS SUBSEQUENT TO INJECTION OF NONANTIGENIC SUBSTANCES AND SPECIFIC ANTIGEN INTO BLISTER

The cantharidin blister forms by the collection of tissue fluid within the malpighian layer of the skin. The cavity thus created contains a liquid portion and formed elements which we have described in the first part of this study. We were able to demonstrate that the number of eosinophiles in the blister fluid, though varying in different conditions, showed little variation in separate blisters in the same individual and was not subject to spontaneous changes. It seemed, therefore, of interest to inject nonantigenic substances and specific antigens into blisters in which eosinophiles were absent and to observe their ability to induce eosinophilia by withdrawal of fluid in regular intervals subsequent to the injection.

Our knowledge about the chemotaxis of eosinophiles is limited (McCutcheon[19]). Ingraham and Wartman[20] have shown, however, that eosinophiles are attracted in vitro by the same substances which attract neutrophiles. They move rapidly toward *Staphylococcus aureus*, more slowly toward peptone (adsorbed on titanium dioxide), least actively toward *Trichinella spiralis* (dried and ground).

Histologic findings and experimental evidence point to the existence of other chemotactic factors specific for eosinophiles, the nature of which is as yet unknown. Since eosinophilia develops as the result of an antigen-antibody reac-

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tion in the anaphylactically sensitized animal, it seemed worth while to investigate the effect of substances which, such as histamine (Code,[21] Dragstedt[22]), are known to be instrumental in anaphylaxis. In addition, we have studied the action of cell-free fluid from blisters rich in eosinophiles on noneosinophilic blisters and that of the injection of ragweed extract in noneosinophilic blisters of ragweed-sensitive persons.

Experimental Procedure.—Fourteen nonallergic persons and six patients with respiratory allergies, sensitive to ragweed, participated in the study. None had more than 1 per cent eosinophiles in the cantharidin blister prior to the experiment. The eosinophiles in the differential blood count varied in the nonallergic group from 0 to 4 per cent, in the allergic group from 0 to 6 per cent. The ragweed-sensitive patients gave strongly positive skin reactions to the injection of ragweed extract 0.0001 mg. N per cubic centimeter and had circulating antibodies demonstrated by passive transfer. Cantharidin blisters were produced, four on each individual, by the method previously described. After initial withdrawal of fluid for differential counts, 0.1 c.c. of the substance under investigation was injected directly into the blister. During removal of the injecting needle, collodium in ether was applied with a medicine dropper to the surface of the blister to seal the opening. Subsequent specimens of blister fluid for differential counts were withdrawn thirty minutes, one hour, two hours, and three hours after injection.

The following substances were injected: (a) histamine phosphate,* 1:1000 representing 0.36 mg. histamine base per cubic centimeter; (b) filtrate of fluid from cantharidin blisters of two patients with urticaria who had a differential blister count of 25 and 28 per cent eosinophiles, respectively. After withdrawal, the blister fluid was transferred into a micro-Seitz filter of 2.0 ml. capacity and centrifuged for fifteen minutes at moderate speed. The filtrates were clear and cell free and represented approximately one-half of the original volume; (c) ragweed extract, 0.001 mg. of total N per cubic centimeter.

The nonallergic group represented a total of fifty-six blisters of which fifty-one were suitable for experimental purposes. Of the fifty-one blisters, thirty-two were injected with histamine phosphate, twelve with the filtrate of blister fluid rich in eosinophiles, and seven blisters were not injected to serve as controls.

The allergic group represented a total of twenty-four blisters of which twenty-one were suitable for experimentation. Of the twenty-one blisters, eleven were injected with ragweed and six with histamine phosphate. Four blisters were not injected and served as controls.

Results.—

Gross Changes: The injection of histamine in blisters of nonallergic and allergic individuals and the injection of ragweed extract into blisters of ragweed-sensitive patients produced extensive erythema which developed not later than ten minutes after injection and surrounded the site of the injected blister. It persisted for a period of from fifteen minutes to one hour and was accompanied by a marked tissue edema recognized by the rapid increase in size of the injected

*Burroughs Wellcome & Co., New York, N. Y.

blister. It is of interest that no itching was noted by any but two individuals even during the most pronounced reactions. The injection of filtrate of blister fluid caused no visible changes.

Microscopic Findings: In spite of the macroscopic response to the injection of histamine and specific antigen into cantharidin blisters, the differential cell count of the blisters remained unaltered. At the end of three hours, the highest percentage of eosinophiles observed in blisters injected with histamine was 2 per cent; with ragweed extract, 2 per cent. Forty-nine of the total of seventy-two blisters showed no change in differential count regardless of the number of eosinophiles in the peripheral blood or of the intensity of the vascular reaction. The injection of cell-free filtrate of blister fluid rich in eosinophiles had no influence on the differential count of blisters in which eosinophiles were absent.

Discussion.—The failure of nonspecific substances such as histamine, but especially of specific antigen, to produce eosinophilia in cantharidin blisters of allergic patients might be due to various reasons. It is possible that the injected material had no positive chemotactic effect on eosinophiles. The vascular reactions which followed the injection of histamine and ragweed extract suggest, however, that even if these substances were to induce chemotaxis in eosinophiles, such an effect might not become apparent in the blister itself. The results of the injection of ragweed illustrate the likely sequence of events. The cantharidin blister by its location within the malpighian layer is not part of the anaphylactic shock tissue of the skin. The union of antigen and antibody might take place in the blister fluid, but the subsequent tissue changes which result (for example, in the liberation of histamine) are probably a function of the subjacent cutis. Studies on guinea pigs[23] suggested to us that eosinophiles are not attracted by the antigen-antibody reaction itself, but by a factor which originates at the site of the shock tissue involved in such a reaction. Consequently, we must expect that eosinophiles would not enter an existing cantharidin blister upon the injection of ragweed in ragweed-sensitive patients, but that those which were present would leave it and move toward the cutis. By the same token, we must assume that a high eosinophile count in cantharidin blisters consists of eosinophiles which were lodged in the cutis and were carried by tissue fluid into the developing blister space. In other words, the blister reflects passively the changes of the cutis present at the moment of the blister formation.

Kauffmann[13] had demonstrated by a large number of biopsies of cantharidin blisters and underlying cutis that a predominance of lymphohistiocytes in the cantharidin blister is the result of the presence of predominantly mononuclear cells in the cutis, a high neutrophile count of its polymorphonuclear infiltration. Similarly, Goodman[16] states, on the basis of cantharidin blister studies and histologic examination of patients with dermatitis herpetiformis, that "the primary source of provocation for the formation of eosinophiles is in the cutis which is probably the point of attack in this disease."

Substances injected into the blister space diffuse rapidly into the cutis. We have no evidence that any modifications in the cellular components of the cutis which might be caused by histamine would affect the differential cell count of the fully developed blister. In the evaluation of our negative results of the

injection of ragweed extract in ragweed-sensitive patients, the time factor assumes new significance. In guinea pigs sensitized and reinjected with horse serum, the interval between anaphylactic shock and maximal eosinophilic response extends from twelve to twenty-four hours (Hajos[24]).

We must conclude that changes in the differential blister count might be expected only if it were possible to influence the cellular reaction within the cutis prior to the application of cantharides.

PART IIB. EOSINOPHILE COUNT OF CANTHARIDIN BLISTERS RAISED ON NORMAL SKIN ALTERED BY INJECTION OF NONSPECIFIC MATERIAL AND BY PASSIVE SENSITIZATION AND ON ALLERGIC SKIN ALTERED BY REINJECTION OF SPECIFIC ANTIGEN

The cellular reaction of the cutis can be altered in several ways. Tommasini,[12] in his studies on blister formation, had shown that the use of irritant plasters other than emplastrum cantharidis caused significant changes in the differential cell count of the completed blister which he attributed to the presence of chemotactic agents in the irritating substance. Kauffmann[13] reported comparative studies of blisters raised on normal skin to which tincture of iodine had been applied; he found marked differences affecting each of the cellular components of the blister. The experiments of Tommasini and Kauffmann have a common denominator: The reaction which became manifest in the altered differential blister count had been initiated before the development of the blister.

In our own studies, we have tried to influence the cellular reaction of normal cutis by the injection of nonspecific material and by passive sensitization and of allergic cutis by reinjection of the specific antigen.

Experimental Procedure.—

General Considerations: The recognition that the site of injection might determine the character of the differential count in cantharidin blisters makes it necessary to emphasize the fact that intradermal injections are injections into the cutis. Taylor[25] has demonstrated in experiments with India ink that a typical "bleb" infiltrates the corium—that it is impossible, as a matter of fact, to inject into the epidermal layer of the skin.

The choice of a nonspecific substance to be injected into the cutis in the attempt to produce a local eosinophilia raised several theoretical questions which will be discussed later. One of the reasons to expect a positive chemotactic action on eosinophiles of any biologic material is the fact that such material, itself, may contain eosinophiles. Of several substances which fulfill this criterion, we have selected nasal secretion of patients with pollinosis and vasomotor rhinitis.

The material was available to us in sufficient quantity. Its preparation has been described in a previous publication.[27] "Nasal secretion was obtained by insertion into each nostril of cotton plugs saturated with 10% sodium chloride solution; satisfactory secretory response was usually obtained without appreciable irritation within 10 minutes. The material obtained was transferred immediately into 2 micro-Seitz filters each of 2.0 ml. capacity, and pressed

through the filters by centrifuging for 40 minutes at moderate speed. The filtrates were clear, slightly viscous and represented approximately one-fifth of the original volumes."

Sera were obtained from six ragweed-sensitive patients with circulating antibodies. The nonallergic individuals who participated in the experiment gave negative skin tests to intradermal injection of ragweed extract 0.01 mg. N per cubic centimeter. Successful transfer was established by the injection of 0.1 c.c. of ragweed extract 0.01 mg. N per cubic centimeter into the blister raised at the transfer site. We used a more concentrated ragweed solution than in preceding studies on allergic individuals because none but local reactions were to be expected. Normal serum obtained from a nonallergic individual was transferred into control sites.

The injection of specific antigen into the cutis of sensitive individuals requires an adaptation of the concentration used to the sensitivity of the individual under investigation. Only 0.1 c.c. of the selected concentration was injected in order to keep the mechanical tissue damage uniform in all cases. For the same reason, control sites were injected with 0.1 c.c. of diluent used in the preparation of extracts.

Technique: Nine nonallergic subjects and sixty allergic individuals under treatment participated in the experiment.

On nonallergic individuals, four sites were selected, two each on corresponding levels of the flexor surfaces of the upper arms. The first site was injected intradermally with 0.1 c.c. of nasal filtrate; the second, with 0.1 c.c. of normal serum; the third and fourth, each with 0.1 c.c. of serum from ragweed-sensitive patients. For each of the last two sites, however, serum of two different patients was used.

Twenty minutes later, emplastrum cantharidis was applied to the site of each of the four injections. The subsequent procedure was identical to the one described in preceding experiments. Ragweed extract was injected into each of the four blisters. Differential blister counts were obtained prior to the ragweed injection and thirty minutes, one hour, and two hours after the injection.

On allergic individuals, two sites, one on the flexor surface of each upper arm, were selected. One site was injected intradermally with 0.1 c.c. of the extract to which the individual was known to be sensitive. Extracts of molds, dust, ragweed, grass, and tree pollens were used. The concentrations of the extract varied from 0.01 to 0.0001 mg. N per cubic centimeter for pollen extracts, from 1:10 to 1:4000 weight by volume for mold and dust extracts. The selected concentrations produced a marked positive skin reaction in each particular case. The other site was injected with 0.1 c.c. of diluent.

Twenty minutes after the injection of specific extract and diluent, respectively, emplastrum cantharidis was applied to both sites in accordance with the procedure previously described.

Sixteen hours after cantharidin application, fluid was withdrawn and differential cell counts obtained from the blisters on either side; no further studies were carried out on the blisters.

A differential blood count was made of each individual at the time of the first withdrawal of fluid from the cantharidin blister.

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Results.—Of a total of thirty-six blisters raised in nonallergic individuals, thirty-two were suitable for experimentation. Of these sites, nine had been injected with normal serum, nine with the filtrate of nasal secretion, and fourteen with serum of ragweed-sensitive patients. The differential eosinophile count of the blisters in the first group (normal serum) varied from 0 to 3 per cent; in the second group (nasal filtrate), from 0 to 2 per cent; and in the third group (ragweed serum), from 0 to 14 per cent. In the last group, however, only three sites showed a differential count of 8, 8, and 14 per cent, respectively, while the remaining blisters did not exceed 3 per cent. The three sites which showed an increase in eosinophiles were found in three different individuals and had been injected with the serum of two donors. One of the sera caused an increase in eosinophiles in blisters on two different individuals, but the serum of the second donor failed to alter the differential blister count in another individual. The donors had a peripheral eosinophile count of 2 and 5 per cent, respectively. A review of the clinical data gave no indication as to the possible reason for the action of the sera on the differential eosinophile count of the cantharidin blister.

Injection of ragweed extract into blisters with less than 3 per cent eosinophiles caused no significant changes in their differential counts; injection of ragweed extract into the three blisters which had developed eosinophilia caused a decrease to 5, 6, and 5 per cent, respectively, after two hours.

The gross changes, erythema and edema, which followed the injection of ragweed extract into blisters raised in ragweed-sensitive individuals were also observed after injection into blisters raised at passive transfer sites; the vascular symptoms, however, were less intense. They were absent when ragweed extract was injected into blisters which had formed at sites prepared by the injection of nasal filtrate or normal serum.

Sixty allergic individuals were sensitive to molds (18), dust (14), ragweed (19), grass and tree pollen (9); all had respiratory symptoms. Of 120 blisters raised in the entire group, 111 were suitable for interpretation. The percentages of eosinophiles found in blisters and blood of those fifty-two patients who developed comparable blisters at the site of the injection of diluent as well as at the site of injection of specific antigen are listed in Table II.

The results indicate the marked variation which exists in the number of eosinophiles in the differential blister count of allergic patients under treatment. They also show that the changes which occur in such differential counts at the site of previous injection of specific antigen are not uniform. An increase in eosinophiles of 3 per cent or more was observed in blisters at the site of antigen injection in 7 of 16 mold-sensitive patients, 3 of 11 dust-sensitive patients, 9 of 16 ragweed-sensitive patients, and 4 of 9 patients sensitive to grass or tree pollen. A decrease in eosinophiles of 3 per cent or more was seen in 3 of 16 mold-sensitive patients, 1 of 11 dust-sensitive patients, and 3 of 16 ragweed-sensitive patients. The rest of the patients showed a variation of 2 per cent eosinophiles or less between differential counts of blisters raised at the site of injection of diluent and those raised at the site of antigen injection. No connection seemed to exist between cellular changes and the concentration of extracts used. As in previous experiments, the lack of correlation between peripheral eosinophilia and blister eosinophilia became apparent again.

TABLE II. PERCENTAGE OF EOSINOPHILES IN PERIPHERAL BLOOD IN CANTHARIDIN BLISTERS RAISED AT CONTROL SITES AND AT SITES OF ANTIGEN INJECTION IN PATIENTS SENSITIVE TO MOLDS, DUST, RAGWEED, GRASSES, AND TREES

PATIENT	SKIN REACTIVITY TO	PERCENTAGE OF EOSINOPHILES IN CANTHARIDIN BLISTERS RAISED AT		PERCENTAGE OF EOSINOPHILES IN PERIPHERAL BLOOD
		SITE WHERE DILUENT WAS PREVIOUSLY INJECTED	SITE WHERE ANTIGEN WAS PREVIOUSLY INJECTED	
1	Mold 1:400	3	4	0
2	Mold 1:4000	11	5	5
3	Mold 1:4000	1	4	1
4	Mold 1:40000	1	1	5
5	Mold 1:4000	3	7	3
6	Mold 1:400	0	0	0
7	Mold 1:400	11	24	12
8	Mold 1:4000	3	3	2
9	Mold 1:40000	11	4	6
10	Mold 1:400	4	9	2
11	Mold 1:4000	0	5	1
12	Mold 1:4000	18	15	4
13	Mold 1:40000	2	2	3
14	Mold 1:4000	3	2	0
15	Mold 1:400	9	14	0
16	Mold 1:4000	1	4	0
17	Dust 1:100	7	0	2
18	Dust 1:1000	7	11	2
19	Dust 1:10	7	5	10
20	Dust 1:100	2	0	10
21	Dust 1:1000	3	3	6
22	Dust 1:1000	8	14	6
23	Dust 1:100	2	8	8
24	Dust 1:10	0	0	2
25	Dust 1:1000	0	2	4
26	Dust 1:100	0	2	7
27	Dust 1:100	2	5	5
28	Ragweed 0.001	0	5	1
29	Ragweed 0.0001	8	4	1
30	Ragweed 0.01	12	6	5
31	Ragweed 0.001	3	8	5
32	Ragweed 0.001	4	7	2
33	Ragweed 0.001	8	8	1
34	Ragweed 0.01	2	9	2
35	Ragweed 0.01	24	19	9
36	Ragweed 0.001	2	21	11
37	Ragweed 0.01	1	3	11
38	Ragweed 0.01	4	7	4
39	Ragweed 0.001	4	8	0
40	Ragweed 0.001	0	0	0
41	Ragweed 0.001	0	3	9
42	Ragweed 0.01	1	6	6
43	Ragweed 0.001	2	2	1
44	Grasses 0.001	4	4	4
45	Grasses 0.001	0	2	3
46	Grasses 0.01	4	6	2
47	Grasses 0.001	0	2	2
48	Grasses 0.01	12	45	9
49	Trees 0.01	8	14	9
50	Trees 0.001	0	1	3
51	Trees 0.01	3	6	3
52	Trees 0.01	5	8	5

DISCUSSION

The absence of a definite concept for the function of eosinophiles explains the uncertainty of selection and the wide range of substances used in the literature on experimental eosinophilia.

Our reasons for including nasal filtrate in our experiments might illustrate the problem. We have been engaged for some time in studies of the part played by mesodermal elements as mucus in the mechanism of respiratory allergies. In unpublished experiments with Dr. E. L. Becker, we have succeeded in producing a generalized skin reactivity to ragweed in nonallergic individuals by passive transfer of relatively small amounts of nasal filtrate from an untreated highly allergic ragweed-sensitive patient. Transfer in a similar manner of this patient's serum, on the other hand, caused only local sensitization. We do not know which role, if any, can be attributed to nasal filtrate in the interpretation of the phenomenon which we have described. We have been unable to reproduce such results with nasal filtrate of other patients. Our few and irregular positive findings, however, have encouraged us to use the material in parts of our present studies. It seems to us that a statement made by Fleming[26] in a discussion of spreading factors with regard to bacterial infection holds true for allergic manifestations: "We have become so preoccupied with those factors both cellular and humoral, which have been thought to lead to success or failure in combating such infections that the study of the site at which these factors exert their effect has been almost completely disregarded as being of minor significance."

We have none but initial information about the changes which take place in mesodermal elements during the process of sensitization; the affinity of eosinophiles to connective tissue has been repeatedly noted.[25] It seemed of interest, therefore, to investigate whether passive transfer of filtrate of nasal secretion rich in eosinophiles of allergic patients would cause an increase in eosinophiles in the injected cutis.

Our study has demonstrated, on one hand, that either transfer of nasal filtrate or, as a rule, passive sensitization fails to alter the eosinophile count of cantharidin blisters raised at the site of injection in nonallergic individuals. On the other hand, we have shown that it is possible at least in certain instances to modify the number of eosinophiles in cantharidin blisters by assumedly nonspecific means; that is, without preceding antigen-antibody reaction. The reagin titer of the serum which caused the appearance of the eosinophiles in blisters raised at the site of injection might be irrelevant for such effect. A nonspecific constituent might be responsible; we are unable as yet to interpret the factors which have induced the changes which we have described.

The findings on allergic patients under treatment suggest that the cutis is not a uniform shock tissue but subject to local variations of its cellular response. This seems important since we have previously shown that untreated ragweed-sensitive individuals presented only slight variations of the differential count of cantharidin blisters raised at different skin sites. It remains for us to determine the length of time required to restore the disturbed cellular balance of the cutis after antigen injection.

The literature on the number of eosinophiles in human skin of nonallergic and allergic individuals is controversial (Berger and Lang,[28] Knott and Pearson,[29] Kline, Cohen, and Rudolph,[30] Jadassohn[31]).

Kline, Cohen, and Rudolph described a high transitory eosinophilia in the skin of allergic individuals, twenty to twenty-five minutes after injection of histamine or specific antigen, which failed to develop in nonallergic persons. Knott and Pearson, in comparative studies of local and peripheral eosinophilia, maintained, however, that histamine increased the number of eosinophiles in the skin of both nonallergic and allergic subjects: It was found to be twice as high as the peripheral count in the former, two and one-half times as high in the latter. Yet while Knott and Pearson stated that the percentage of eosinophiles at the sites of negative skin tests and in the skin of normal controls equals the percentage of eosinophiles in the peripheral blood, Jadassohn's specimens show local eosinophilia after mechanical irritation as well as after injection of morphine, atropine, pilocarpine, even in the absence of peripheral eosinophilia.

The discrepancies found in the literature might be due in part to the number of variables which complicate the evaluation of allergic individuals. Our own patients presented many such variables as type and duration of sensitivity, environmental exposure, length and frequency of treatment. In addition, however, we found that even in comparable patients the cellular response of the cutis seemed to follow a pattern of its own. Of two female patients with fall pollinosis (of equal age, recent onset of symptoms, comparable titer of serum reagins, pronounced skin reactivity to ragweed extract 0.0001 mg. N per cubic centimeter, a peripheral eosinophile count of 760 and 840 eosinophiles per cubic millimeter, a blister eosinophile count at the site of the diluent of 1 and 2 per cent, respectively) one had 21 per cent eosinophiles in the blister fluid at the site of ragweed injection, while the other failed to show any significant changes. If we accept the presence of eosinophiles as evidence for certain immunologic processes, it would then follow that in one instance the skin participates in such processes while in the other it does not. This is of interest since the positive skin reaction was identical in both patients and, therefore, per se does not attract eosinophiles.

We have attempted to investigate further the conditions under which cutaneous eosinophilia develops. We were fortunate enough to be able to observe consecutive changes in the cell count of cantharidin blisters raised in regular intervals on noninjected skin of a small group (four) of patients treated with Hapamine. Hapamine (histamine conjugated with a despeciated horse serum globulin through azo-linkage), although it fails to stimulate in human beings the formation of antibodies to histamine, is a potent specific antigen. Injections of the 1:100 dilution of the preparation were given in weekly intervals, beginning with 0.1 c.c. and increasing by 0.1 c.c. each subsequent injection. Initial cantharidin blister counts showed a percentage of 1, 4, 4, and 7 per cent eosinophiles, respectively. Two days after the fifth injection all of the patients gave positive skin reactions to intradermal injection of the dilution of Hapamine used for treatment. At this time, two of the patients showed no significant changes in the differential blister count, two showed an increase to 18 and 32

per cent eosinophiles, respectively. The patient who had the highest eosinophile count developed a generalized urticaria after the next or sixth injection of Hapamine.

It might be suspected that the differences in the eosinophilic response which we have noted are due not only to variations in the reactivity of the cutis (other than its reagin titer) of nonallergic and allergic subjects, but also to variations in skin involvement in the immunologic mechanism of each allergic individual. If this is the case, the differential cell count of the cantharidin blister seems to afford a reliable method for their further investigation.

SUMMARY

1. The differential eosinophile count of cantharidin blister fluid showed variations in groups of various diseases. Such variations were independent of the number of eosinophiles in the peripheral blood.

2. Injection of histamine and filtered fluid from blisters rich in eosinophiles into developed blisters of normal individuals and of ragweed extract into developed blisters of ragweed-sensitive patients failed to alter the differential blister counts within three hours after injection. Injection of histamine into blisters of normal and allergic persons and of ragweed extract into blisters of ragweed-sensitive patients caused edema and erythema of the skin surrounding the blister site.

3. Passive transfer of nasal filtrate and of serum of ragweed-sensitive patients into the skin of nonallergic individuals did not, as a rule, induce eosinophilia in blisters raised at the site of injection, although the latter caused an increase in the percentage of eosinophiles in a few isolated instances. In allergic patients under treatment, the differential eosinophile count of blisters raised at the site of previous injection of the specific antigen showed marked variations. In some cases, it was significantly higher than the eosinophile count of blisters raised at control sites in the same individual. Other patients of equal clinical sensitivity, however, failed to show significant changes.

4. The theoretic implications of these discrepancies are discussed.

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DISCUSSION

THEODORE L. SQUIER, Milwaukee, Wis.—Dr. Samter's work demonstrates that eosinophiles in blister fluid are carried in with that fluid as it develops in the blister space.

Injection of specific ragweed antigen into preformed blisters of sensitive patients increased the erythema and edema surrounding the blister site, *but failed to alter significantly the number of eosinophiles in the fluid.* On the other hand, there was a definite rise in the eosinophiles in the fluid of blisters induced during the time of active allergic tissue reaction.

It is apparent, therefore, that blister fluid reflects the transitory localized eosinophilia which occurs in tissues at the site of antigen-antibody reaction as described by Kline, Cohen, and Rudolph for allergic wheals. The evidence from this basic work indicates that local tissue eosinophilia rather than blood eosinophilia determines the number of eosinophiles in the blister fluid. It further suggests the concentration of eosinophiles at the site of the allergic tissue response. The high eosinophile content of blister fluids during acute urticaria and the relatively low count of blister fluids in asthma as compared with blood eosinophile levels is entirely consistent. Only to this extent does it seem justifiable to conclude that there is a characteristic variation in the eosinophile content of blister fluids in various diseases.

ALLERGY IN JAPAN*

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THE status, concepts, and scope of allergy in Japan today present striking contrasts to allergy as we know it in the United States. Numerous differences are evident in environment, types of allergic conditions, and methods of treatment. These differences are apparent from the study of a variety of material. Sources of information were personal interviews with leading Japanese physicians and visits to medical schools, hospitals, and libraries. Also included was an examination of over forty Japanese medical books, reprints, and reviews which were interpreted by a Japanese physician. Individual patient records and reports of series of allergy cases were found in the medical literature.

On the main Japanese Islands there are seven Imperial University Medical Schools. The case reports of allergy patients were written for the most part by physicians associated with these schools. In addition there are about eighteen other medical schools which are either smaller, private, or have a relatively short course of instruction. Incidentally, many of the latter do not even require a high school education prior to admission. The Japanese physicians who were consulted personally were either chiefs of large medical services or members of teaching staffs at the Japanese Imperial Medical Schools in Osaka and Kyoto. The literature available to Japanese physicians seemed to be entirely in German or Japanese. A notable exception was Tuft's *Clinical Allergy*.

The data obtained in this study were widely varied in nature and necessarily incomplete. The extensive published material available to the Japanese medical profession is in sharp contrast to their inadequate use of this material in actual practice. No active allergy clinics were seen in operation. Even though Japanese physicians are aware of the influence of environment, of the theory of specific sensitivity, and of treatment by hyposensitization, the clinical application of this knowledge appeared to be most limited.

Differences in environment were most striking and presented marked contrasts to our environment in the United States. These differences are in the topography of the country with its relation to plant life, individual home surroundings, and diet.

In considering first the general nature and topography of the country we find some pertinent facts. The main Japanese Islands have a total area of about 140,000 square miles. On these Japanese home islands live 70,000,000 people as contrasted to the United States, where 140,000,000 people live on about 3,000,000 square miles of land. Thus the area of Japan is almost the same as the area of the state of Montana, or one-twentieth the total size of the United States.

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*Read at the Fourth Annual Meeting of the American Academy of Allergy, St. Louis, Mo., Dec. 15-17, 1947.

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Approximately 50 per cent of Japan is forest land. Thirty-five per cent of the remainder is mountainous and unsuitable for plant life. Only the remaining 15 per cent is under cultivation. One-half of this cultivated area consists of rice fields. Most of Japan has a rugged terrain and steep slopes which set definite limits to the tillable land. Erosion from the high rainfall which is generally prevalent further prevents the development of a productive soil. The cultivated areas may be divided according to their location. Just below the rough, mountainous terrain is the first upland cultivated area which is unterraced. This area grows small grains and grasses. The next lower upland section is terraced and here are produced potatoes, tree fruits, tea, rice, mulberries and vegetables. The next area is the important lowland area in which there is a richer alluvial soil suitable for rice, wheat, barley, and, on the southern portions, citrus fruits. These alluvial soils in the lowlands produce the bulk of the food for the population. Thus, it is readily seen that there are but limited areas suitable for the growth of ragweed. In fact, there was no evidence available from an agricultural standpoint to indicate that grass or ragweed were important factors causing allergic conditions.

The second major factor to be considered is that of home surroundings. House dust does not appear to be an important cause of allergic symptoms among the Japanese. The elements which we consider as contributing to the formation of house dust are to a great extent entirely lacking. If we assume that house dust is an end product of the deterioration of cotton, wool, feathers, kapok, and animal danders, then an examination of Japanese homes finds these materials essentially absent. A typical Japanese living room has no drapes, rugs, pillows, upholstered furniture, or animal pets. These rooms thus approach our concept of a dust-free environment. The bedrooms do not have beds or mattresses. Cotton quilts are placed on the floor and the small pillows are frequently of straw. During the daytime these quilts are folded up and placed in bureau drawers or cupboards. There is usually considerable air circulating through the houses due to their construction and thus house dust cannot accumulate easily.

A study of case reports indicates that sensitivity to dust is rarely considered. Most series of tests do not include house dust nor is it used in treatment to any extent. Three separate reports covering over 200 patients with asthma mention only a few sensitive to house dust extract. However, Japanese physicians are aware that house dust is an important factor in causing allergic conditions in this country. Thus with house dust and ragweed practically nonexistent in Japan the risks of developing nasal or respiratory allergic symptoms are considerably decreased.

Japanese homes are cold in winter compared wtih our American homes. The main source of heat is usually from charcoal which is burned in open braziers. As a result the Japanese are consistently exposed to lower temperatures. Cases of angioneurotic edema are mentioned as being due to exposure to cold.

Turning now to diet, as the third major factor, we find that the most frequent allergic condition recognized in Japan is urticaria. This is far more

common than hay fever or asthma. Reports indicate that urticaria was attributed to foods even before there was any knowledge of the allergic state. The most common foods believed to cause urticaria are pork, milk, eggs, lobster, crab, mackerel, tuna, and bonita. Another discussion of urticaria indicated that it has been associated with liver function, spastic intestinal tract, and constipation. A series of fifty cases of urticaria have been reported with the following positive tests: horse meat, milk, crab, lobster, beef, bonita, tuna, sardines, spinach, *Aspergillus niger*, oyster, and bluefish. Treatment for urticaria was mostly of a nonspecific nature.

Seasonal hay fever due to ragweed is not mentioned in the Japanese medical literature. Possibly ragweed has merely not been introduced in Japan, though the 15 per cent of cultivated land is worked so intensively that hardly any plant life grows that is not of an edible nature. The shaded forest areas and the cold rocky, mountainous regions are apparently unsuitable for the growth of ragweed. No pollen counts were recorded. Case histories indicate that when Japanese developed hay fever in the United States due to grasses or ragweed the symptoms subsided after their return to Japan. These cases were not skin tested and no specific cause or seasonal relationship was noted. Plantain is found on Okinawa and Formosa only. The important and most common trees found in Japan are birch, ash, poplar, elm, oak, and sorrel. No skin tests with these pollens had been done.

Professor Tsuji of the Osaka Imperial University Medical School has done the most work on asthma in Japan. Among his cases there was one patient with asthma due to the inhalation of silk worm cocoon. He also reported an instance of asthma following infestation with Ancylostoma duodenale which cleared after elimination of the parasite. Dr. Nishuma tested a series of asthma patients with 160 allergen extracts and reported that 90 per cent were positive to Ascaris. He tested eighty subjects with asthma with 100 allergen extracts using Coca's solution, and he found positive tests to eggs, milk, salmon, tuna, mackerel, sardine, beef, pork, horse meat, strawberries, spinach, bamboo shoots, tomatoes, dust, and mold. In a number of instances he found that exposure to the foods produced attacks and elimination or avoidance resulted in relief of symptoms. Professor Katsunuma of the Nagoya Imperial University Medical School skin tested eighty patients with asthma. He reported that 48 per cent were positive. Of this group 24 per cent were positive to molds, 18 per cent to foods, 13 per cent to animal epidermis, 5 per cent to bacteria, 5 per cent to plants, Cryptomeria, rubber, and drugs. Dr. Suzuki tested forty-three patients with asthma. Forty-six per cent had positive tests. Five per cent of these patients were positive to one allergen, 11 per cent were positive to cat, bacteria, milk, and insulin, and 18 per cent were sensitive to burdock.

There are numerous articles of diet in Japan that are rarely used in this country. Among these are a wide variety of seafood including shark, whale, carp, octopus, and snails. Roots, millet, herbs, ferns, fungus, and bamboo shoots are also eaten.

There is evidence in the Japanese literature that the allergists are familiar with specific hyposensitization treatment. On the other hand, considerable em-

phasis is placed on nonspecific therapy. In treating with specific allergen extracts they use dilutions considerably smaller than ours. Suggestions are made to take 0.1 c.c. of a concentrated extract and dilute one million times and then dilute this one million times. There are numerous methods of nonspecific treatment. The literature states that the purpose of this treatment is to produce local swelling and a rise in temperature so that complement and antitoxin will be produced. Whole milk is used either subcutaneously or intramuscularly beginning with 1 c.c. to 5 c.c. every five days. There are also Japanese and German casein preparations called Eryosan, Kaseinol, Caseosan, and Aoran. Vegetable protein preparations are also used. Other preparations are peptone, horse serum, autogenous blood, and tuberculin. Combined allergen mixtures are also used containing horse serum, animal lipoids, and bacterial products. Injections of protein from carp are also frequently used. These preparations are marketed under the names of Erstin, Multi, Omnin, and Ogardin. Serous fluid withdrawn from the pleura, abdomen, and joints is used in treatment. Purulent discharges sterilized with heat and mixed with chloroform, Ringer's, and Lugol's solution are also used in treatment.

Vasomotor rhinitis occurs in Japan and two series of cases were found in the literature. Dr. Takahashi examined and reported seventy-nine patients with vasomotor rhinitis of which sixty-two were males and seventeen were females. He used thirty-seven different extracts in testing. Fifty-one patients had a family history of other allergies, such as asthma, urticaria, eczema, and migraine. Professor Fuzizawa reported twelve subjects with vasomotor rhinitis with 70 per cent having hereditary allergic factors.

SUMMARY

These observations indicate that allergy in Japan is in striking contrast to allergy in the United States. The climate and general topography of the country are such that grasses and ragweed are not important factors in causing allergic symptoms. Physicians in practice outside of medical schools do not use, as a rule, specific methods of hyposensitization. Nonspecific therapy is more generally available and more widely practiced. Urticaria is the most prevalent allergic condition. No records of seasonal hay fever were noted. House dust is not an important cause of allergic symptoms. Eggs, milk, wheat, and chocolate are not commonly eaten, while fish and rice are the staple articles of diet among the greatest number of Japanese. Adequate medical literature on allergy is available but the clinical application of this knowledge is limited.

CLIMATOTHERAPY IN THE TREATMENT OF ALLERGIC DISEASES

A Clinical Report*

F. B. Schutzbank, M.D., Tucson, Ariz.

CLIMATIC environment is recognized as one of the most important factors influencing health. It has long been recognized that climatotherapy is of value in the treatment of many allergic diseases. Yet, there is no agreement as to just what produces the improvement when a patient changes climate. Physical or atmospheric factors usually considered to have important influences include temperature, humidity, barometric pressure, ion content of the air, altitude, changeableness of the weather, storminess and sunshine, and type of foliage. Just how any one or combination of these influences such diseases as bronchial asthma is not readily explainable. Empirically it is true, however, that many sufferers are relieved when they move to the subtropical regions of the southwest such as Tucson, Arizona. This report is made on my clinical observations of 100 consecutive allergy patients who were observed for one year or more unless improvement occurred before that time.

When a patient is promptly relieved of asthma or other allergic disease after the change of climate it is likely that he left behind something to which he was allergic. That something may be atopic, as pollen or animal dander, or some environmental factor having a nervous or psychic influence that was the trigger mechanism in exciting the symptoms. Frequently unsatisfactory home conditions, especially in children, are important considerations. The allergic symptoms may be the manifestation of a subconscious rebellion or protest in the life of an allergic individual. Patients who have left their families and business troubles behind may do well in their new surroundings where they can rest and relax and perhaps gain a new outlook on life.

People who are susceptible to climatic variations, storminess, high humidity, and cold will naturally improve in a calm, dry, and warm environment. Tucson has an elevation of 2,400 feet above sea level, an unusually stable barometric pressure, very low average humidity, and a high mean temperature with little rainfall and an abundance of sunshine. The foliage of the Southwest is quite different from that of the East and Middle West. While many patients lose their hay fever, some develop hay fever for the first time, and others have more severe hay fever than they had at home. Due to the nature of the climate the pollen season lasts nine to ten months. Those sensitive to Bermuda grass especially, as well as to some of the tropical pollens, suffer the most. Due to the dryness, there is much dust and that must be considered in dust-sensitive individuals. Also the climate is conducive to inspissation of mucus and in some asthmatics is responsible for bronchial plugging.

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Patients with respiratory tract infection (as determined by examination of the discharges) and a frank bacterial allergy fare the best after a sufficient length of time has been allowed for the infection to clear. Too many patients expect a quick cure from some long-standing condition. At least one year should be allowed before an unfavorable opinion can be drawn in any case. Patients should be advised to receive the treatment usually indicated in their case and not to expect the climate alone to produce a miraculous cure. I have given penicillin by injection and aerosol to over seventy-five patients with respiratory tract infection and the benefits were frequently maintained in the new climate where recurrences were more common with the same treatment in an unfavorable environment. Many of the patients with respiratory tract infection who did not get along well were those who had had extensive or radical sinus surgery with an incurable sinusitis and bronchitis and often bronchiectasis.

In the analysis of the 100 cases herewith reported, sixty-seven were primarily asthmatic patients, twenty-two had allergic rhinitis and sinusitis usually complicated with secondary infection, and eleven were patients with primary skin allergy including eczema, neurodermatitis, and urticaria. Naturally many cases came under more than one classification.

Of the whole group 76 per cent were benefited or relieved by climatotherapy and 24 per cent were not appreciably helped. Of the sixty-seven patients with asthma, fifty-one were completely relieved or benefited by an estimated 50 per cent or more amelioration of symptoms. Of the fifty-one relieved, the improvement could be attributed to avoidance of psychosomatic influences in twenty cases and to improvement of respiratory tract infection in nineteen cases. Escape from some specific atopic offender accounted for improvement in six cases and miscellaneous or undetermined reasons in six cases. In considering these percentages one must bear in mind that most of these patients had received the usual type of treatment at home without benefit before being advised to try climatotherapy.

In the cases attributable mainly to psychogenic causes I do not mean to infer that sensitivity to various allergens played no part at all. I have observed that patients definitely sensitive to certain atopic substances could not tolerate the offenders when they were having asthma. But when they were removed from the emotional disturbing influences they had no asthma and could then tolerate the previously offending foods or inhalants. These individuals have an underlying inherited allergic constitution which manifests itself during periods of nervous or mental strain. When they are helped by change of climate they are merely getting away from the disturbing causes of their nervous or emotional instability. Many individuals are potentially allergic and, as demonstrated during World War II, develop symptoms when under strain.

Of the sixteen patients with asthma not benefited, five cases were considered due to failure to determine or relieve some psychogenic cause and eight patients had chronic respiratory tract infection such as sinusitis, bronchitis, or bronchiectasis that was not amenable to treatment. Other failures were in part due to such complications as emphysema or cardiac pathology. Not included in

the series were six patients with active tuberculosis, discovered on routine sputum examinations of asthmatic patients, one patient with previously undiagnosed carcinoma of the lung with asthmatic symptoms, and four with cardiac decompensation; all of these patients were sent to Arizona for bronchial asthma.

DISCUSSION

DR. BEN Z. RAPPAPORT, Chicago, Ill.—I should like very briefly to remind those who may have read what Dr. Welker, Dr. Nelson, and I said in 1932 and 1933 concerning the causes of the precipitation of asthma during weather changes. We said at that time that in two successive years during the ragweed-hay fever season we placed patients in pollen-free rooms, the second year especially under well-controlled experimental conditions, before the onset of the hay fever season. We controlled the pollen count in the wards so that there was practically no pollen—one or two pollen grains per cubic yard per twenty-four hours. The relative humidity was controlled to either 40 or 50 per cent, I do not recall which. However, it was kept constant. The temperature was controlled by special apparatus at 68 to 70° Fahrenheit. The patients were kept in the wards except for toilet privileges. Twelve patients were in one ward and six in the other.

Early in September, with the occurrence of a rainstorm and a fall in barometric pressure during a period when the pollen count was very low, 50 per cent of the patients in each ward developed asthma. In one case that I recall, despite the fact that the patient was kept in the pollen-free room, the asthma continued for twenty-one days and was very severe.

Since then we have tried to simulate the atmospheric changes that might cause asthma. We have tried introducing ozone into the rooms. We have tried to simulate electrical storms with special apparatus, without, however, any effect on patients with asthma. Dr. Welker is still working on the problem on the lines that perhaps pollen becomes dissolved in the water vapor and that patients inhale water vapor containing pollen in solution.

In addition to discussing the effect of weather, Dr. Schutzbank mentioned psychosomatic influences on his patients. I should like to refer to Dr. Franz Alexander, a well-known psychiatrist on this subject.

At a recent seminar at the University of Illinois, Dr. Franz Alexander presented the viewpoint of the psychiatrist regarding the importance of emotional factors in the causation of asthma. Dr. Alexander emphasized that our present state of knowledge does not permit us to decide the relative significance of either emotional or physical factors. Asthma is not a nosologic entity. The relative importance of emotional factors and of physical factors may differ in other patients with the same symptoms. Each individual case must be evaluated separately. He expressed regret that some physicians are inclined to climb on the bandwagon of psychosomatic medicine without sufficient study of each case.

Let us consider Dr. Schutzbank's twenty patients who were supposed to have symptoms due to psychosomatic causes. They were apparently a very unhappy group of people while at home since all of them, when removed from their homes, uniformly showed improvement that Dr. Schutzbank attributes to removal from family influences. All of them are rather peculiarly selected cases. Apparently all twenty patients (and there were five others that he mentioned) were relieved of allergic symptoms because of their removal from home and placement in the environment of Arizona.

I doubt whether adequate psychiatric study can be given twenty cases in one year's time while carrying on in addition a normal program of practice in allergy—and this, too, without more than a general practitioner's training in psychiatry.

I believe we should be very careful in attributing symptoms to emotional disturbances. I believe that emotional causes may produce or aggravate allergic symptoms but I do not believe we are equipped to do anything except cooperate and present our somatic view to the psychiatrist, and have the psychiatrist present his psychiatric views, unless we become better trained in psychiatry than most of us are now.

Original Articles

DISSIMILAR SENSITIVITY TO ANTIGEN OF SEGMENTS FROM THE ISOLATED INTESTINAL STRIP*†

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INTRODUCTION

SINCE the work of Dale[1] it has been recognized that the uterine horn or intestinal strip from a sensitized guinea pig will contract when brought into contact with the specific antigen in vitro under suitable conditions.

This phenomenon has been utilized in several types of studies: for the analysis of composite biologic antigens[2, 3] and a determination of the antigenic relationship between various vaccines and the normal components of developing chick embryonic tissues in which they were prepared;[4] to investigate the interrelationships of sera;[5] to test the effect of desensitization to one antigen on sensitization to another;[6] to investigate the relation between the effect of histamine and anaphylactic shock;[7] and finally, in an indirect approach to the mechanism of anaphylaxis by attempts to determine the relative inhibition by amino acids[8] and antihistaminic drugs[9] of contractions due to histamine and to antigen.

Two assumptions have been made in these studies: (1) that the sensitivity of the isolated uterine horn or intestinal strip parallels that of the intact animal; that this is true has been claimed by Kendall and Shumate[10] and by Kabat and Landow[11] but disputed by Winter;[12] (2) that the two horns of the uterus or the several intestinal segments (if not taken at too great a distance from the caecum) of a given sensitized animal are of practically equivalent sensitivity; such an assumption is necessary to the validity of the conclusions in most cases.

Whether or not this latter assumption is true should be answerable from the literature. In many investigations, however, one horn or one segment has been used as control and some preliminary treatment given to the tissues subsequently tested so that no comparison of the effect of antigen alone is possible. Occasionally two or more muscle segments have been treated identically. It may be noted that Campbell and Nicoll,[7] using two ileal segments from a sensitized guinea pig, obtained contractions of 0.6 and 1.0 cm., respectively, to 0.0125 μg per milliliter of histamine, and corresponding contractions of 0.8 and 1.3 cm. to ovalbumin. In another study by Nicoll and Campbell,[6] however, contractions of similar magnitude were obtained with several segments from the same animal although slight quantitative differences may be measured in the tracings. In an investigation of the response of sensitized strips to certain

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types of haptenes, Campbell and McCasland[13] mention "differences sometimes shown in strips from the same animals." It is of interest also that they state there is little, if any, difference in anaphylactic responsiveness between intestinal muscle and uterine strips.

EXPERIMENTAL

Intestinal strips were rapidly removed from sensitized guinea pigs killed by a blow on the back of the neck. The strips were cleaned and refrigerated for at least two hours in Tyrode's solution. A segment, 1.0 to 1.5 cm. in length, was then suspended in a bath of 20 ml. volume containing Tyrode's solution plus 0.5 gamma per milliliter of atropine; oxygen was slowly bubbled through the bath which was maintained at 37° C. After fifteen minutes the response of the strip to histamine phosphate was tested. By suitable attachment to a heart lever, contractions of the muscle were recorded on a smoked-drum kymograph. After each addition of histamine the bath was flushed with preheated Tyrode's solution.

After the segment was shown to be capable of reacting to an amount of added histamine not in excess of 1.0 gamma, 0.5 ml. of a 1:10 dilution of egg white was added to the bath. Antihistaminic drugs were allowed to remain in contact with the muscle for forty-five seconds before antigen was added. In all additions to the bath, care was taken to avoid direct contact of the undiluted material with the segment and the syringe was rinsed from the bath several times to ensure mixing.

In the results the quantities of histamine are expressed as the amount of the base added to the bath. S1 in the tables represents the segment nearest the caecum. On different occasions S1 or S5 was used first so that the additional time of refrigeration prior to use was apparently not a significant factor in the results.

The guinea pigs were sensitized by the intraperitoneal injection of 1.5 ml. of rabbit anti-egg white serum (titer, 1:10,000) twenty-four or forty-eight hours before use.

RESULTS

In Table I it may be seen that the amount of contraction was not constant when the same quantity of antigen was added to the bath containing successive segments from the same sensitized animal. In some instances no contraction occurred with one or two segments although the remainder reacted. In a few instances the contraction after addition of antigen was very slow (up to seven minutes).

In these experiments, no attempt was made to achieve maximum sensitivity of the muscle to histamine before the effect of antigen was tested. If a measurable response was obtained to 1.0 gamma or less of histamine, the egg white solution was added. In Table II is shown a comparison of the response of the segments from certain animals to a constant amount of histamine, with their response to the antigen. It appears that the degree of sensitivity of the muscle to histamine is not an important factor in the extent of contraction due to antigen.

TABLE I. COMPARISON OF DEGREE OF SENSITIZATION OF SEVERAL ILEAL SEGMENTS FROM THE SAME GUINEA PIG AS MEASURED BY HEIGHT OF CONTRACTION

GUINEA PIG	S1 (CM.)	S2 (CM.)	S3 (CM.)	S4 (CM.)	S5 (CM.)
1	5.5	0	0	4.9*	--
2	7.5	5.7	1.7	0	2.6*
3	2.6	4.2	4.2	0.2	0
4	6.6	7.0	5.4	10.0	9.2
5	--	--	0.9	1.2	6.8
6	1.9	4.4	3.1	3.8	--
7	2.8	6.5	0	4.3	4.1

-- Indicates segment was not tested.
*Slow response to antigen.

Before it was realized that the portions of a strip from a given animal were apparently not equally sensitive to antigen, attempts were made to determine the effect of various antihistaminic drugs on the extent of contraction. In one such experiment 0.2 gamma of Neoantergan was used: with one segment there was no contraction on the addition of antigen; with four other segments responses equivalent to 25, 31, 50, and 58 per cent, respectively, of that of the control strip were obtained. In a few instances the contraction of a segment treated with an antihistaminic drug was even greater than that of the control. These findings are further evidence that the sensitivity varied from segment to segment of muscle from a given animal.

TABLE II. LACK OF CORRELATION BETWEEN DEGREE OF RESPONSE TO 1.0 GAMMA OF HISTAMINE AND TO ANTIGEN AS MEASURED BY HEIGHT OF CONTRACTION

GUINEA PIG	REACTION TO	S1 (CM.)	S2 (CM.)	S3 (CM.)	S4 (CM.)
2	H*	1.5	--†	0.8	1.1
	A*	7.5	--	1.7	0
6	H	1.5	3.1	0.4	1.0
	A	1.9	4.4	3.1	3.8
			(S3)	(S4)	(S5)
7	H	0.9	0.9	0.6	1.9
	A	2.8	0	4.3	4.1

*H indicates 1.0 gamma of histamine added; A indicates antigen, egg white, added.
†Before antigen was added 0.5 gamma of histamine was used; contraction to antigen was 5.7 cm., as shown in Table I.

SUMMARY

1. In guinea pigs passively sensitized to egg white, it has been found that the resulting sensitivity of the isolated intestinal strip to the antigen varied from segment to segment.

2. Since the muscle becomes desensitized on contact with the antigen, and therefore the same segment cannot be used to test the response to antigen with and without prior drug treatment, it appears unsatisfactory to use the isolated intestinal strip for the determination of drug activity against the effects of the antigen-antibody reaction.

The authors wish to acknowledge the technical assistance of Mrs. Muriel B. Smeeton.

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LEVELS FOR PYRIBENZAMINE AND BENADRYL IN BLOOD AND URINE FOLLOWING A SINGLE ORALLY ADMINISTERED DOSE*

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SEVERAL workers have called attention to the great tendency for N^1-pyridyl-N^1-benzyl-N-dimethyl-ethylenediamine monohydrochloride (Pyribenzamine†) to cause gastrointestinal upsets and for beta-dimethylamineothyl benzhydryl ether hydrochloride (Benadryl‡) to produce a preponderance of symptoms affecting the sensorium.[1-3] Moreover, "side effects" from Benadryl frequently appear more rapidly than with Pyribenzamine, although they are not as long sustained.[3] The present studies were undertaken to determine whether these differences in effect might be accounted for, at least in part, by the rate of absorption (as indicated by blood levels attained) and by the amount of drug excreted unchanged in the urine during a period of twenty-four hours.

METHODS AND MATERIALS

Five men and five women, selected at random from the wards of a general city hospital, served as subjects. It was predetermined that none of them was suffering from allergic disease or from recognizable abnormality of the autonomic nervous system. Their ages varied from 29 to 67, with an average of 45.8 years. Tests of hepatic and renal function failed to show any dysfunction of the liver or kidney in any instance.

The determination of Benadryl and Pyribenzamine was carried out for blood and urine according to the method for determining secondary amines as devised by Brodie[4] and later modified.[5] Each subject was tested for his responses to each of the two substances. One week was allowed to elapse between the tests. For one week prior to the first test and until the second was completed the diet was controlled, particularly in regard to the quality and amount of protein and the total caloric equivalent.

For the test with each drug, 400 mg. were given orally in a single dose as a suspension in 50 c.c. of water after the patient had fasted for not less than ten hours and had had no water to drink for not less than four hours. Prior to taking the drug and at one-half hour intervals thereafter for three hours, specimens of 5 c.c. of blood were withdrawn from the antecubital veins for the determination of the amount of drug present.

The patient voided just before the ingestion of the antihistamine compound. This specimen was discarded. All urine subsequently voided for twenty-four hours was measured and the amount of compound identifiable by the modified Brodie test[5] was estimated.

*From the New York Medical College, Metropolitan Hospital Research Unit, Welfare Island.

†Pyribenzamine for these studies was furnished by Dr. R. L. Mayer of Ciba Pharmaceutical Products, Inc., Summit, N. J.

‡Benadryl for these studies was furnished by Dr. E. A. Sharp, of Parke, Davis & Co., Detroit, Mich.

RESULTS

Determination of Blood Levels for Benadryl and Pyribenzamine.—In micrograms per cubic centimeter, the levels for Benadryl are shown in Fig. 1 and those for Pyribenzamine in Fig. 2.

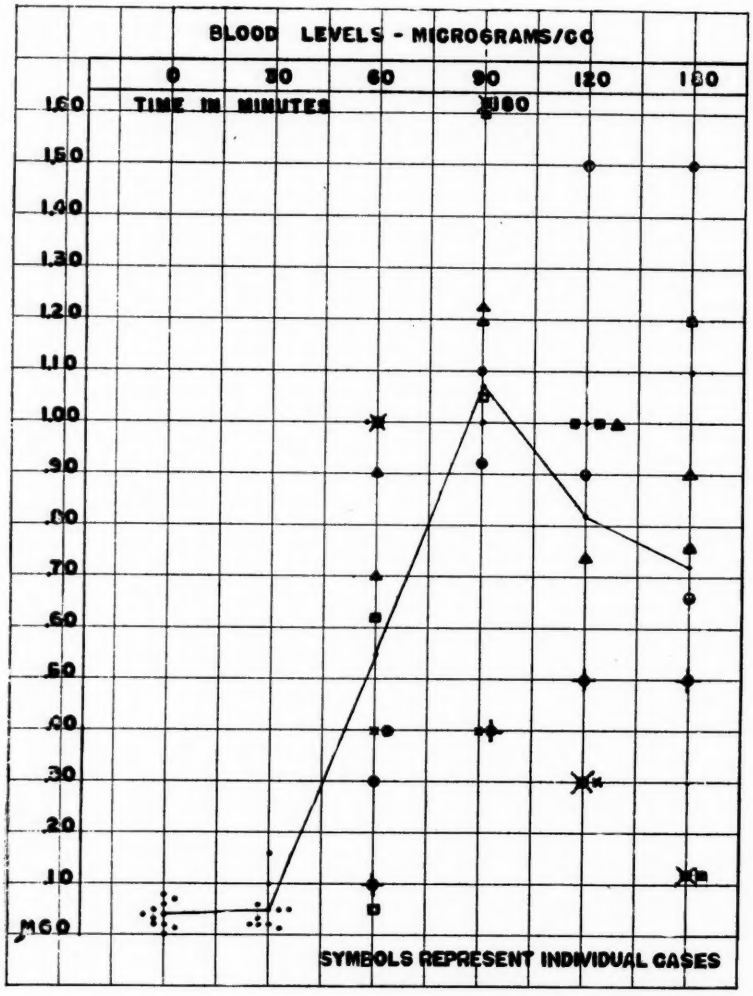

Fig. 1.—Basic amines—Benadryl.

While there is considerable "scattering" above and below the line of averages, a peak in the blood value for Benadryl is attained at ninety minutes, with an appreciable change within the first hour. In some instances the highest value occurred as early as sixty minutes or as late as the second hour. Peak values ranged from 0.50 to 1.80 μg per cubic centimeter. Because of the wide variations from sixty minutes onward, the figures observed for each individual who received Benadryl have been plotted with identifying symbol (Fig. 1).

Seven of the ten subjects who were given Benadryl showed an unpleasant reaction to the drug, which was invariably observed between the first and second hours and lasted for from one to two hours. In five of these, drowsiness was the predominant feature, and in two dizziness was most noticeable.

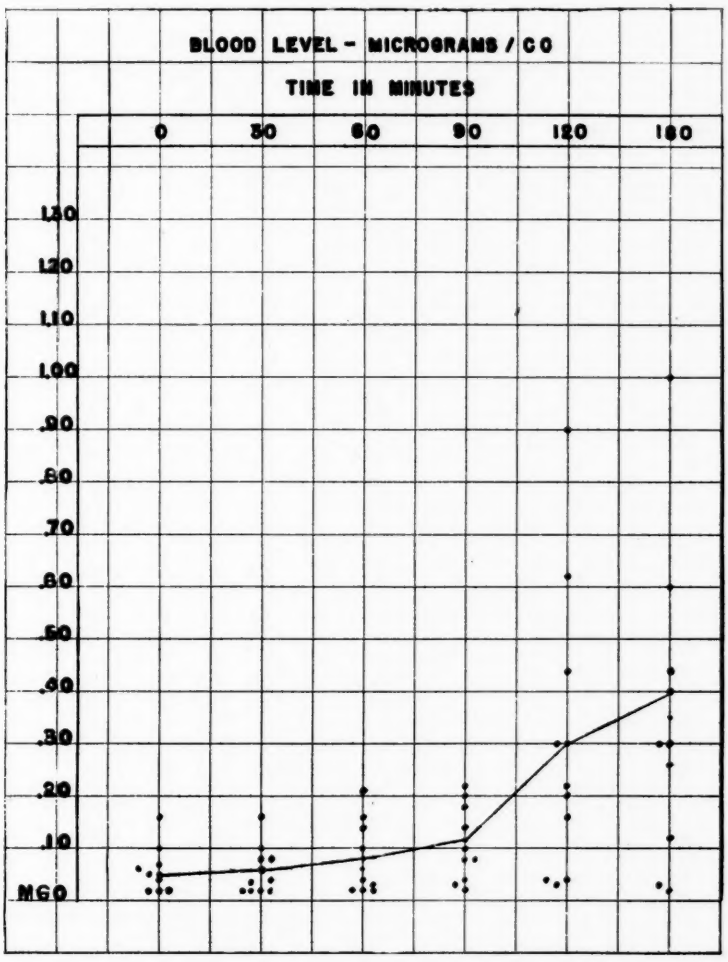

Fig. 2.—Basic amines—Pyribenzamine.

With Pyribenzamine the "scattering" was not so marked (Fig. 2). The rise in blood values was much slower, becoming appreciable only after two hours, and sometimes not reaching its peak by the end of the third hour. Indeed, in three of the ten subjects the value for secondary amines was still in the range of the pretreatment levels at the end of the third hour.

In seven of the ten subjects Pyribenzamine produced some untoward reaction. In six of these severe nausea and dizziness were the prominent manifestations. In the seventh, dizziness and blurring of the vision were present. In five subjects, data from which are not included in the present report, it

was necessary to abandon a comparison between the absorption rates of the two drugs because of the vomiting produced when the subject ingested Pyribenzamine. In three of these the Benadryl portion of the test had been completed without difficulty; in the other two, no effort had been made to give Benadryl.

Values for Benadryl and Pyribenzamine in the Urine.—The average urinary excretion of Benadryl in six subjects for twenty-four hours following a single oral dose of 400 mg. was 184.1 mg., or 46 per cent of the ingested dose, with a range from 132 to 270 mg. or from 33.0 to 67.5 per cent. For Pyribenzamine the corresponding averaged excretion in five subjects was 80.5 mg., or 20.1 per cent, with a range from 63 to 102 mg. or from 15.8 to 25.5 per cent.

DISCUSSION

The test used for making these determinations is obviously not specific for either of the drugs under investigation. However, rather consistent values were found in the fasting specimens for other amines of the blood capable of giving the reaction, so that while the absolute figures may have little or no value, the changes in them following the ingestion of each of the antihistamine compounds are undoubtedly significant. In the urine the absolute values are probably more representative than in the blood. Nevertheless the amounts excreted may be materially influenced by the pH of the urine. The excretion of other similarly reacting substances and the degradation of the compound itself will also alter the results.

It appears that at least part of the difference in the untoward reactions caused by Benadryl and Pyribenzamine can be accounted for on basis of a difference in absorption and excretion. Benadryl is absorbed rapidly so that gastric irritation is minimal, but the resulting high blood levels account for early and severe sensorial symptoms, particularly drowsiness. The delay in absorption following the ingestion of Pyribenzamine is probably secondary to its predominantly spastic influence upon the duodenum as compared with the relaxing and quieting action of Benadryl. Therefore, the present observations offer at least one explanation for the slower and much less dramatic, although more prolonged, action of Pyribenzamine.

SUMMARY

1. Blood levels and urinary excretion of Benadryl and Pyribenzamine, respectively, were determined in each of ten subjects following a single dose of 400 milligrams.

2. Values in the blood for Benadryl began to rise within sixty minutes and reached an average peak of 1.07 micrograms per cubic centimeter between 90 and 120 minutes. With Pyribenzamine an appreciable rise was first evident at 120 minutes and the trend was still upward at 180 minutes.

3. Within the first twenty-four hours 46.0 and 20.1 per cent of the ingested Benadryl and Pyribenzamine, respectively, were excreted in the urine.

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STUDIES ON HISTAMINE AND HISTAMINE ANTAGONISTS*

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THE problem of the mechanism of anaphylactic and allergic reactions has been studied by many investigators. In recent years considerable evidence has been accumulated to support the histamine theory of these manifestations and reviews have been published by Feldberg,[17] Dragstedt,[14, 15] Rocha e Silva,[32] and Rose.[36]

Several established facts have suggested that histamine constitutes a potent factor, although not an exclusive one, in the production of the symptoms of anaphylactic shock in some animals. The evidence involving histamine in allergic diseases in human beings has been less conclusive although a number of investigators have presented some evidence that histamine plays a significant role in allergic manifestations.

The recent discovery and preparation of new drugs possessing marked antihistaminic and antianaphylactic actions have resulted in general interest, not only in their clinical applications, but also in the possibility of establishing, through their activity, the role of histamine in allergic reactions.

The purpose of this paper is to report briefly the results of our experiments on histamine and histamine antagonists.

Histamine

LIBERATION OF HISTAMINE DURING WHEAL FORMATION

An attempt was made to detect a local release of histamine by means of repeated determinations of blood histamine during the production of allergic skin reactions, since an increase of blood histamine might be due to a release of histamine from a stimulated area.[51]

Methods.—The blood histamine was determined by the Code modification[9] of the method of Barsoum and Gaddum.[4]

In previous observations on a group of thirty-nine normal individuals[20] it was shown that 5.88 γ per 100 c.c. is the average histamine content of whole blood with variations of from 3.80 to 7.98 γ per 100 cubic centimeters. In all our publications, histamine values have been expressed as gamma (gamma or γ = 0.001 μg. or microgram) of histamine base per 100 c.c. whole blood.

The experiments can be divided in three groups as follows:

(1) Fifteen to twenty allergic wheals were produced by scratching the skin of the forearms of thirteen subjects suffering from hay fever with pollens to which they were sensitive. Blood samples were taken five to ten minutes after the stimulation.

(2) Six patients with dermatographia were scratched for three minutes in an area extending from the nape of the neck to the waist. With this technique the local whealings were much greater than the previous ones. Besides, the

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samples of blood for the estimation of histamine were taken before and repeatedly within the first few minutes after the stimulus was initiated.

(3) In one patient suffering from bronchial asthma (horse dander) fifty-four wheals were produced by means of intradermoreactions with horse dander extract and then took blood samples one, three, five, seven, eleven, fifteen, and twenty-five minutes after the stimulus was initiated. Besides, in order to establish, at least approximately, the amount of histamine released from the allergic reactions, the blood histamine was determined in the same subject before and a few minutes after a subcutaneous injection of histamine (1 mg. histamine dichlorhydrate) and fifty-four intradermal injections of histamine (2 mg. of histamine dichlorhydrate).

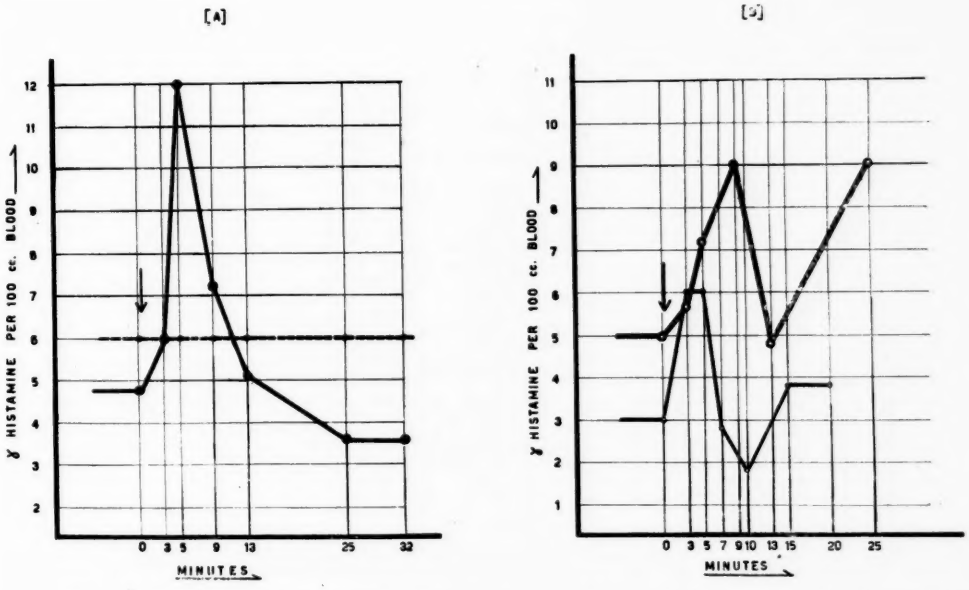


Fig. 1.—Blood histamine determinations in a case of horse dander bronchial asthma (30-year-old male). *A*, Black line shows changes of the blood histamine following fifty-four intradermal injections of horse dander extract (0.1 c.c. of 1:10,000 dilution for each injection). A production of marked wheals and general symptoms occurred. Broken line shows the control: blood histamine after fifty-four intradermal injections with saline solution. *B*, Changes of blood histamine in the same patient following the histamine injections. Light line; 1 mg. of histamine dichlorhydrate was injected subcutaneously. Heavy line; 2 mg. of histamine dichlorhydrate diluted in 5.4 c.c. of saline solution were injected intradermally in fifty-four sites.

Results.—In the first group of experiments an increase of blood histamine, after the application of the stimulus, was observed only in two subjects; a decrease was noted in one subject, and no significant change in the others.

In the second group, an increase of blood histamine was observed in five of six patients with dermatographia. It will be noted that the increase of blood histamine appeared one to two minutes after the application of the stimulus and that the blood histamine had usually fallen to a normal level after three to five minutes.

In the third group, a well-marked increase of the blood histamine followed by a decrease was observed (Fig. 1, A). Some general manifestations (flushing

of the face, asthma) also occurred in the patient. A similar increase of the blood histamine was noted after the administration of histamine by subcutaneous and intradermal injection. This increase was not so marked as that obtained during the production of allergic wheals.

Discussion.—These observations are in agreement with those of Rose.[34] On the other hand, Katz[25] tested for the liberation of histamine during local allergic reactions in the skin.

It is felt that three factors are important for the evidence of histamine release in circulating blood; namely, the extent of the skin manifestations, the time of blood sampling, and the different rate of histamine disappearance from the blood of these patients.

From these experiments it may be concluded that histamine is released during allergic wheal production and disappears from the circulating blood in a very short time.

HISTAMINE TOLERANCE CURVE

The results of the determinations of blood histamine in allergic patients during acute symptoms and in the quiescent status often have been conflicting and do not explain the relation between histamine and allergic diseases. It was thought that the histamine tolerance curve in allergic patients as compared with normal subjects could clarify this problem.[45, 56]

Methods.—The blood histamine was determined before one, two, three, five, seven, ten, fifteen and twenty minutes after the subcutaneous injection of 1 mg. histamine dichlorhydrate. This was done because in previous observations on cases of dermatographia histamine was observed to disappear from the blood in a very short time (one to two minutes).

The results in normal persons and in allergic patients are illustrated in Fig. 2.

Results.—It was noted that there is a difference in the response of the blood histamine following a subcutaneous injection of histamine in allergic patients as compared with normal subjects. In eight normal subjects either a decrease or no change was noted. In fourteen asthmatic patients a definite increase occurred after a few minutes. The content of blood histamine usually fell to a normal level in a very short time. Sometimes a secondary decrease to below the original level was observed. In one subject the test was repeated three times in one year and a marked increase of blood histamine was always observed followed by a decrease. Each time the histamine injection caused an attack of asthma in this patient.

The increase of blood histamine after histamine injection in nine patients with hay fever and vasomotor rhinitis, in nine patients with urticaria, and in other allergic conditions was not so constant as in the asthmatic patients. However, in most cases we observed an increase of blood histamine. In the others no significant change or a decrease was noted. In these cases, however, we took the blood specimens only five times after the injection, at intervals longer than the previous ones.

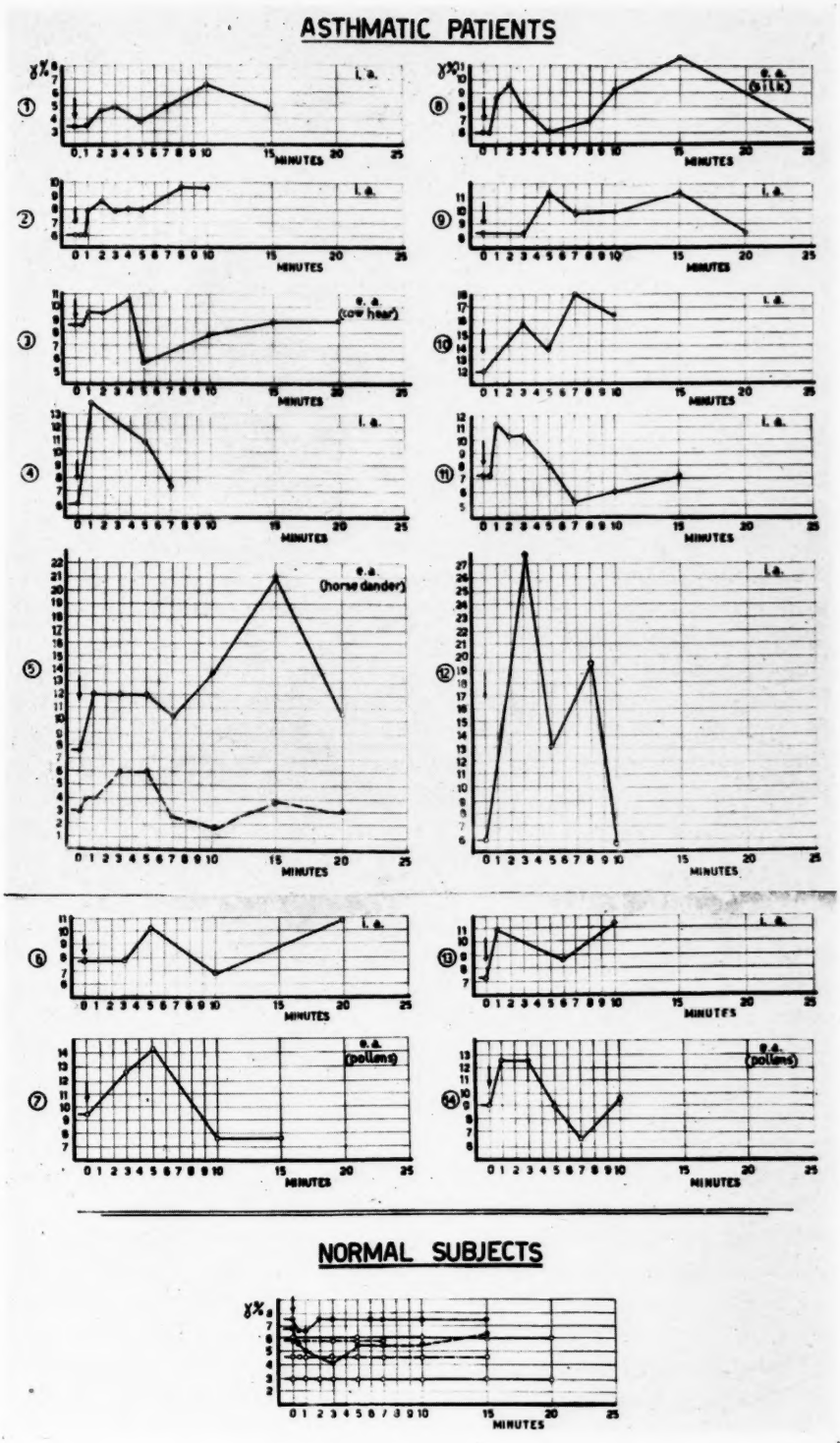


Fig. 2.—Variations of blood histamine following a subcutaneous histamine injection (1 mg. histamine dichlorhydrate) in asthmatic patients and in normal subjects. *i. a.*, Intrinsic asthma; *e. a.*, extrinsic asthma. In Case 5, two trials performed at six-month intervals are represented.

It will be recalled that subcutaneous injection of 1 mg. of histamine dichlorhydrate in allergic patients results in the production of a group of symptoms (an attack of asthma in asthmatic patients generalized urticaria in patients with urticaria, collapse, etc.). During these reactions the content of blood histamine varied; it was either increased, normal, or reduced. In the latter an increase preceded the decrease.

Discussion.—From the results of these experiments it appears that the histamine tolerance curve establishes a difference betwen the normal subjects and the asthmatic and other allergic patients, which, as far as we know, has not been noticed before. In normal subjects there are important antihistaminic mechanisms so that no change or decrease of blood histamine results. In the allergic patients these antihistaminic mechanisms are not efficient so that in the great majority of cases the content of blood histamine increases, sometimes for a very short time. The rapid fluctuations of blood histamine, as constantly seen in the asthmatic patients (Fig. 2), can explain the conflicting results of many observers of blood histamine during allergic reactions.

Rose,[35] studying the variations of blood histamine during the allergic manifestations produced by histamine injection, observed in ten cases a decrease of blood histamine. This is presumably due to the time of drawing of blood specimens. Also, in many of our cases the blood histamine was reduced or normal ten to fifteen minutes after histamine injection and during the ensuing manifestations.

The variations of blood histamine are not easy to explain. The increase may be produced by the injected histamine which is not destroyed by the tissues of these subjects in which Albus[1] demonstrated a decrease of normal histaminase activity. It is possible, on the other hand, that the injected histamine releases more histamine. Some investigators, Lunedei[27] and Jiménez Diaz,[22] suggested that the fundamental character of allergic diseases is the facility to release histamine substance in the shock organs following the action of different stimuli. In some cases we found that the amount of histamine in the blood after the injection was greater than that injected. This fact and the great rate of the increase of blood histamine suggest that the last hypothesis may be more probable.

The decrease of blood histamine observed during the allergic reactions is in agreement with the observations of Rose[35] and other experimenters on human beings, and of Code,[11] on horses and calves. The mechanism is not clear. These results simply show that histamine does not accumulate in the peripheral blood. It is possible that the tissues affected may absorb the free histamine and withdraw it from the circulating blood. In allergic wheals, Kline, Cohen, and Rudolph[26] obtained results showing an increase of the eosinophiles which, according to Code, carry most of the histamine. It was demonstrated that the histamine decrease during anaphylactic shock in rabbits is due to accumulation in the pulmonary circuit of the white blood cells which, in these animals, are very rich in histamine (Code and Ing[13]). It is important to know whether the histamine which is in the blood during allergic manifestations is in the cells or in the plasma. Code's results[10] showed a marked increase of histamine in the plasma during anaphylaxis in the dog and in the guinea pig. The antigen-

antibody reaction producesa shifting of histamine from cells to plasma, in vitro.[24, 25] Staub[55] recently observed a release of histamine from the cells into the plasma after an intravenous injection of adrenalin in a human being. The total histamine content of the blood was not increased. It is possible therefore to have an increase of histamine in the plasma without modifications of total blood histamine. This can explain some allergic or histaminic reactions in man which occur without increase of total blood histamine.

Some treatments can modify the histamine tolerance curve.

1. *Histamine Tolerance Curve After Fever Therapy in Asthmatic Patients.*[48]—Recently the effects of fever artificially produced on the histamine tolerance curve and the symptoms of bronchial asthma caused by injection of histamine were studied.

In a patient suffering from bronchial asthma, in whom the injection of histamine always produced an increase of blood histamine and an attack of asthma, the Ducrey vaccine was administered by intravenous injection and a typical febrile reaction was obtained. During the febrile reaction an increase of blood histamine was again noted and a histamine injection brought about a new and very severe attack of asthma. On the following day (the patient having no fever) we injected histamine again for a new tolerance curve and observed that the content of blood histamine was reduced in all the determinations. After this last injection, asthma did not occur.

2. *Histamine Tolerance Curve After Injection of an Antihistaminic Drug.*[50]—In eight asthmatic patients in whom the injection of histamine produced an increase of blood histamine and an attack of asthma, the experiment was repeated after injecting 2 c.c. of a 2 per cent solution of dimethyl-aminoethyl benzylaniline (antergan). In every patient no significant change of blood histamine was observed and no asthma occurred in these cases. The antihistaminic drug presumably present in the extracts ready for the titration was withdrawn by Walthert's method.[57]

THE BLOOD HISTAMINE AFTER PHYSICAL EXERCISE

In two patients with urticaria due to overexertion[51] a well-marked increase of blood histamine was found after physical exercise which produced the urticarial manifestations. It was interesting to study the blood histamine after physical exercise in normal subjects and in allergic patients.[47] Experiments were carried out with three normal subjects and four patients suffering from hay fever by making them go through the following exercise: (Riccioni) getting up from a horizontal position to the maximum vertical extention. This was repeated every four seconds. The patients within about two minutes were very exhausted. Several blood specimens were taken for histamine determinations before and a few minutes after the end of the physical exercise. In normal subjects no significant change of blood histamine was observed. In patients suffering from hay fever in quiescent status (winter) an increase of blood histamine was found which occurred immediately in two patients and ten minutes following the physical exertion in two patients. In one of these patients who suffered during the season from rhinitis and asthma we observed

an attack of asthma. This attack was severe for twenty minutes and slight for the following two hours. The blood histamine which had increased before the beginning of the attack fell to below the original level in a very short time. The experimental results are illustrated in Fig. 3.

I wish to draw attention to the fact that a nonspecific stimulus (physical exercise) produced in a specifically sensitive (ragweed) patient a well-marked increase of blood histamine and an attack of asthma.

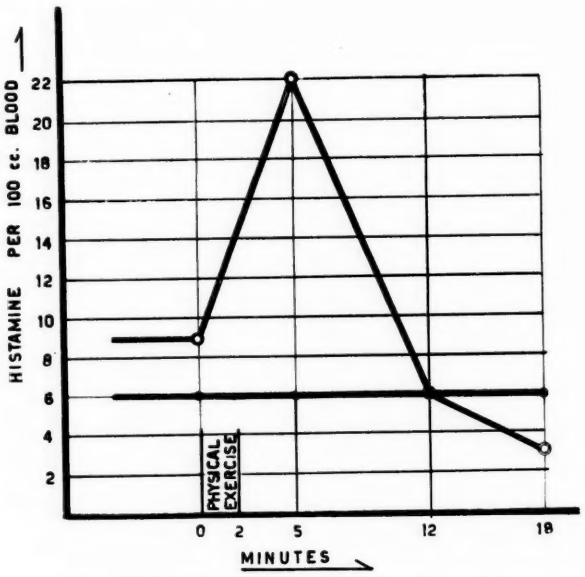


Fig. 3.—The blood histamine before and a few minutes after physical exercise. Lower line, in a normal subject; upper line, in a patient with hay fever. An attack of asthma occurred in the latter patient.

The increase of blood histamine after physical exercise in patients suffering from hay fever and the attack of asthma observed in one of these subjects suggest that the symptoms produced by physical exercise in other allergic patients may be due to the increase of blood histamine. It is probable that the clinical manifestations appear only in patients whose tissues are sensitive to histamine. But, we observed in these and in previous experiments that the increase of blood histamine is not always followed by allergic reactions.

Our reported experiments on histamine seem to indicate that it plays a significant role in allergic reactions and in allergic diseases. Many observations, however, suggest that histamine is not the exclusive factor in allergic manifestations. Many substances (choline, acetylcholine, substance P Gaddum, slow reacting substance) not yet well studied must be considered.

It is interesting to note that many stimuli (antigen-antibody reaction in the skin, histamine injection, physical exercise) produce in allergic patients an increase, followed by a decrease, of blood histamine.

Finally, it should be kept in mind that the blood histamine we studied only reflects the cellular changes as a consequence of which the histamine-like substances are liberated in the blood.[12]

Histamine Antagonists

THE EFFECT OF ANTIHISTAMINIC DRUGS ON HISTAMINE AND ANTIGEN-ANTIBODY SKIN REACTIONS IN HUMAN BEINGS

Oral Administration.[39-43]—Dilutions of histamine and allergens covering a relatively wide range were chosen. A series of intradermal tests was made on the upper part of the back of patients, by injecting 0.1 c.c. of histamine solution in normal subjects and 0.1 c.c. of allergen extracts in patients clinically sensitive to various allergens (pollens, horse dander). Fifteen minutes later the outlines of the reactions were traced on transparent paper. The diameters of both wheals and erythematous flares were measured. All patients were then given, orally, antihistaminic drugs. In these experiments the following were used: 40 mg. of dimethyl-amino-ethyl benzylaniline (Antergan or compound 2339 R.P.*); 40 mg. of N-p methoxybenzyl-N-dimethylaminoethyl aminopyridine (Neoantergan or compound 2786 R.P.†); 10 mg. of N. dimethylaminopropyl-thiodiphenylamine (compound 3277 R.P.‡); 30 mg. of 2-(N-phenyl-N-benzyl-aminomethyl)imidazotin (Antistin§); and 150 mg. of beta dimethylamino-ethyl benzhydryl ether hydrochloride (Benadryl‖). The experiments were performed on thirty-four patients.

In order to establish the time effect of the drugs, the subjects were retested with the same histamine and allergen extract, exactly one, two, four, eight, and twenty-four hours after the ingestion of the antihistaminic drugs. The results were again traced and compared to those obtained before the drug administration. As a control, the tests were done on the same subjects without administering the antihistaminic drugs to determine the influence of repeated injections on either histaminic or allergic skin reactivity. No significant difference could be detected.

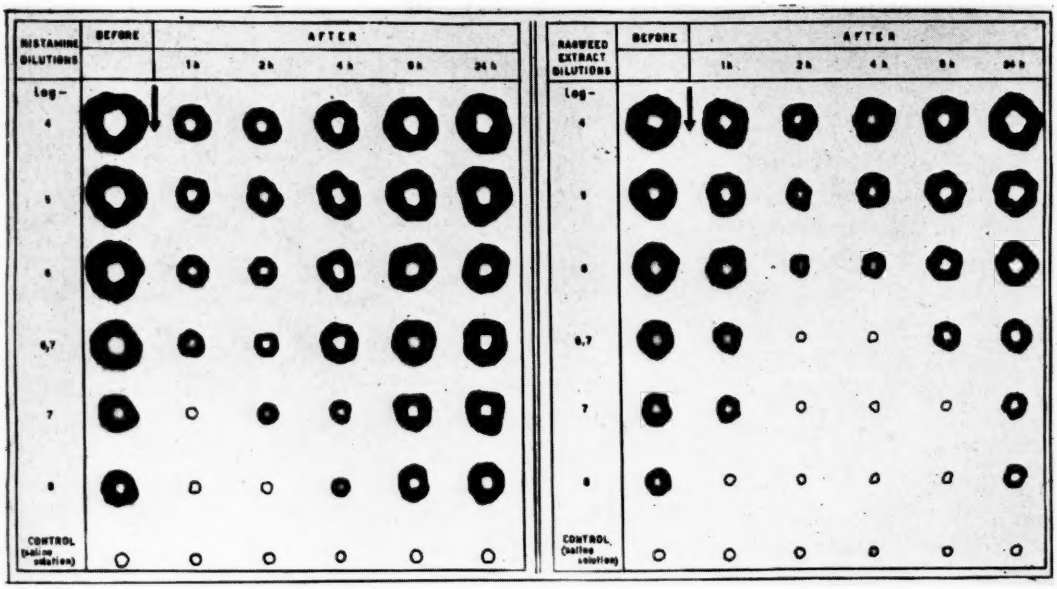
The tracings of histamine and allergic wheals obtained in two patients before and after the antihistaminic drugs are shown in Fig. 4.

The antihistaminic drugs studied have a definite and constant effect on histamine and allergic wheals, as observed in 1945.[39] The effect is constant from two to four hours after oral administration. The findings therefore agree with those of most workers.[6, 7, 15, 21, 28, 29, 37, 56, 58]

The inconsistent results obtained by some investigators[2, 19] may be due to the dose of the different antihistaminic drugs administered or to the fact that these men studied the effect of these substances only forty-five minutes to one hour after the administration.

The findings obtained with this technique show that the maximum effect does not always occur at the same time. Frequently it is not complete after one hour. This is probably due to the different absorption rates of adequate amounts of antihistaminic drugs through the gastrointestinal canal, as Arbesman[2] also suggested. Additional support is shown by the following experiments.

*The drug (Dimetina) was supplied by S. A. Lepetit Milano.
†Furnished through the courtesy of Dr. Durel, Rhône-Poulenc.
‡Furnished through the courtesy of Dr. Halpern.
§Supplied through the courtesy of Ciba S. A. Bâle.
‖Furnished through the courtesy of Mr. Sorieri of U.N.R.R.A.



A. *B.*

Fig. 4.—*A*, Effect of a single oral dose of Benadryl (150 mg.) on size of reactions produced by serial dilutions of histamine in a normal subject. *B*, Effect of a single oral dose of Benadryl (150 mg.) on size of reactions produced by serial dilutions of allergens in a hay fever patient (grasses).

Intravenous Administration.—Similar experiments were performed on seventeen patients by intravenous injection of some antihistaminic drugs (Antergan, Neoantergan, Antistin). A constant and definite decrease of size of histaminic and allergic reactivity was noted earlier than after oral administration. The tests were done before and 20, 40, 60, an 120 minutes after the injection. The maximum effect of the drug was shown forty minutes after the administration. No significant change was observed two hours later.

Prolonged Treatment.—An inhibition in the histaminic and allergic reactions was observed after four and seven days of prolonged oral treatment. The tests were performed at least eight hours after the administration of the last dose. After the drug had been discontinued for two days the skin reactivity returned to its previous status.

It may be concluded that the antihistaminic drugs by oral and intravenous administration produce a constant and definite decrease of histaminic and allergic skin reactions. The inhibition is most discernible in the flares and to a lesser extent in the wheals. The effect is most marked two to four hours after oral administration and forty minutes after intravenous injection.

The decrease of skin reactivity produced by antihistaminic drugs is not so striking as to render doubtful the results of diagnostic allergic skin reactions.

OTHER EXPERIMENTAL RESEARCHES

Dimethyl-amino-ethyl benzylaniline (Antergan), by intravenous injection (2 c.c. of 2 per cent solution), does not inhibit the rise of histamine-induced

gastric acidity[48] and does not modify the blood sugar. This substance, as stated, inhibits asthmatic symptoms and the increase of blood histamine produced by histamine injection in asthmatic patients.

It was also noted that this drug by intravenous injection produces a constant increase of blood potassium and an inconstant decrease of blood calcium in normal and asthmatic subjects.[53] These variations were marked in asthmatic patients fifteen to thirty minutes after the injection.

While histamine produces a decrease in the hemolytic capacity of concentrated saline solutions, the antihistaminic drugs (Antergan, Neoantergan, Antistin, Benadryl) show a definite inhibition.[54]

The antihistaminic drugs also show an antibacterial activity. The growth of *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Salmonella paratyphoid A* and *B*, and *Bacillus melitensis* in a liquid medium is inhibited by Dimetina (or Antergan), Antistin, and Neoantergan at 200 to 2,000 γ per ml. dilutions.[38]

CLINICAL RESULTS WITH DIMETHYL-AMINO-ETHYL BENZYLANILINE (ANTERGAN)

Dosage and Administration.—The average adult dose varied from 40 to 80 mg. daily. The substance was in most of the cases given orally and usually in two daily divided doses during meals. In some cases the drug was administered by intravenous injection. Many patients have taken this drug daily for as long as one year or more, without untoward effects.

Relief of symptoms appears often within one-half hour. The duration of the effect of the drug showed considerable variation as shown by the previous reported experiments on the effects of antihistaminic drugs on histaminic and allergic skin reactions.

Other antihistaminic drugs were also used, as Neoantergan, Antistin, and Benadryl. These substances are somewhat different chemically. However, no definite difference was noticed in their clinical efficacy. The inadequate number of cases studied does no permit us to draw any significant conclusion at present.

Results of Treatment.—One hundred forty patients presenting a variety of allergic symptoms are included in this study. A summary of the effect of the drug on the symptoms of various allergic diseases appears in Table I. The effect of the drug was classified as complete when all discomfort was relieved; as partial when only some of the discomfort was ameliorated or the advantage was inconstant; no relief when all the discomfort persisted.

The allergy was entirely palliative and temporary, generally lasting from three to six hours. A number of patients were relieved on some occasions and had no relief under similar conditions at another time.

Urticaria responds to antihistaminic drugs better than any other allergic condition. The itching is usually relieved first and then the skin manifestations disappear. The results are sometimes dramatic.

In bronchial asthma the results were not so striking as in the previous group, as seen in Table I. The drug seems to be more effective prophylactically

than therapeutically. Extrinsic bronchial asthma is benefited most. The attacks of bronchial asthma due to histamine injection were always prevented by intravenous injection of antihistaminic drug, as already stated.

TABLE I. SUMMARY OF CLINICAL RESULTS WITH DIMETHYL-AMINO-ETHYL BENZYLANILINE (ANTERGAN)

DIAGNOSIS	NUMBER OF CASES	SYMPTOMATIC RELIEF						SIDE REACTIONS	
		COMPLETE		PARTIAL		NO BETTER			
		CASES	%	CASES	%	CASES	%	CASES	%
Urticaria									
Acute*	31	29	93.5	1	3.3	1	3.2	9	29.0
Chronic*	17	12	70.6	3	17.6	2	11.8	6	35.3
Factitia	4	3	75.0	1	26.0	0	–	1	25.0
A frigore	1	1	–	–	–	–	–		
Total	53	45	84.9	5	9.4	3	5.7	16	30.2
Bronchial asthma									
Extrinsic	9	4	44.5	3	33.3	2	22.2	2	22.2
Intrinsic	36	8	22.2	16	44.5	12	33.3	11	30.6
Total	45	12	26.7	19	42.2	14	31.1	13	28.9
Hay fever	15	10	66.7	5	33.3	0	–	2	13.3
Vasomotor rhinitis (perennial)	6	4	66.7	2	33.3	0	–	1	16.7
Pruritus	7	5	11.4	1	14.3	1	14.3	0	–
Contact dermatitis	2	1				1		2	100.0
Atopic dermatitis	5	3		2	40.0			1	20.0
Drug eruptions (sulfa)	3	2		1	33.3	0		0	–
Migraine	3	1		1	33.3	1		0	–
Serum allergy	1	1		0	–	–		–	–
Total	140	84	60.0	36	25.6	20	14.4	35	25.0

*This group also includes patients with angioneurotic edema and dermographism.

Many patients suffering from hay fever and vasomotor rhinitis showed definite benefit; two cases of the latter showed dramatic improvement following the treatment.

It is interesting to note that constitutional reactions (asthma, urticaria) in the course of routine hyposensitization therapy disappeared in a very short time when the drug was given. For this, we usually give the drug during the hyposensitization therapy, especially in those patients in whom repeated constitutional reactions developed during the treatment. It was possible to reach high doses of the allergen extracts. Recently Arbesman[3] obtained similar results.

The clinical results here described are in agreement with those of other workers who studied the effect of antihistaminic drugs on allergic diseases. The voluminous literature in this field is reported in recent reviews.[5, 8, 18, 30, 31, 41]

Side Reactions.—Dimethyl-amino-ethyl benzylaniline is apparently harmless in effective doses. Some untoward, although not serious, side effects were observed. It was found necessary to discontinue the drug because of severity of side reactions in only eight patients treated.

The symptoms attributed to the medication were, in order of frequency, nausea, general discomfort, headache, rise in temperature, gastric pain, dizziness, exhaustion, diarrhea, muscular pains, insomnia, anorexia, drowsiness, vomiting, stupor, nervousness, collapse, early menses.

An aggravation of allergic symptoms was noted in three cases. An attack of bronchial asthma occurred in two patients with chronic urticaria.

CONCLUSIONS

1. The blood histamine rises after skin stimulation in sensitive patients. This is presumably due to a release of histamine from allergic skin reactions. Three factors are important for this evidence: the extent of the manifestations, the time of blood sampling, and the different rates of histamine disappearance from the blood.

2. A difference in the response of blood histamine following a subcutaneous injection of histamine (histamine tolerance curve) was noted in allergic patients as compared with normal subjects. An increase of blood histamine, followed frequently by a decrease, was observed in the great majority of allergic patients. Normal subjects showed either no change or a decrease in blood histamine. It was possible to establish these variations only by means of repeated determinations of blood histamine at very frequent intervals.

3. Some treatments, as fever therapy and histamine antagonists, can modify the histamine tolerance curve in allergic patients. The increase of blood histamine following the histamine injection did not occur in asthmatic patients after fever therapy or the injection of antihistaminic drugs. In these experiments the asthmatic symptoms produced by histamine injection did not appear.

4. Physical exercise produced an increase of blood histamine for a very short time in some allergic patients (hay fever). A rather severe attack of asthma occurred in one patient and a well-marked increase of blood histamine was observed. The blood histamine after the same physical exercise showed no significant change in normal subjects.

5. The antihistaminic drugs inhibit constantly histaminic and allergic erythematous flares and wheals. The maximum effect occurred two to four hours after oral administration. Frequently it is not complete after one hour but this is probably due to a different absorption rate of antihistaminic drugs through the gastrointestinal canal. The maximum effect was observed forty-minutes after the intravenous injection of antihistaminic drugs.

The histamine antagonists which showed this activity were dimethyl-amino-ethyl benzylaniline (Anergan or Dimetina), N p. methossy benzyl N dimethyl-amino-ethyl aminopyridine (Neoantergan), N. dimethylaminopropylthiodiphenyl-amine (3277 R.P.), 2-(N-phenyl-N-benzyl-aminomethyl)imidazotin (Antistin), beta dimethylamino-ethyl benzhydryl ether hydrochloride (Benadryl).

6. Other researches showed that dimethyl-amino-ethyl benzylaniline does not inhibit the rise of histamine-induced gastric acidity and does not modify the blood sugar. Dimethyl-amino-ethyl benzylaniline and 2-(N-phenyl-N-benzyl-aminomethyl)imidazotin produce by intravenous injection a constant increase of blood potassium and an inconstant decrease of blood calcium in normal and asthmatic subjects. Several antihistaminic drugs (Antergan, Neoantergan, Antistin, Benadryl) inhibited the hemolytic capacity of concentrated saline solutions. Histamine, on the contrary, produced a decrease. Some antihistaminic drugs also showed an antibacterial activity.

7. Clinical studies with dimethyl-amino-ethyl benzylaniline were made on 140 allergic patients. The drug completely relieved 84 of 140 patients (60 per cent). The best results were obtained in acute and chronic urticaria, pruritus, and hay fever. Undesirable, although not serious, side effects were observed in 45 of 140 patients (25 per cent).

It may be concluded that our experiments afford further evidence that histamine plays an important, although not exclusive, role in allergic conditions in human beings.

SUMMARY

1. The results of some experiments on histamine and antihistaminic drugs in allergic patients were reported.

2. A histamine release from allergic skin reactions was shown.

3. A disturbed histamine metabolism was shown in allergic patients by an increase of blood histamine after histamine injection (histamine tolerance curve) and physical exercise. No significant change or a decrease was noted in normal persons under similar conditions. Some treatments (fever therapy, antihistaminic drugs) inhibit the increase of blood histamine and the allergic symptoms produced by histamine injection in allergic patients.

4. In regard to histamine antagonists a constant inhibition of allergic reactions after oral and intravenous administration of these drugs was observed. Other experimental researches were reported.

5. Significant symptomatic relief in many allergic manifestations was obtained in 84 of 140 allergic patients (60 per cent).

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ANTIHISTAMINE DRUGS IN HAY FEVER*†

A Comparative Study With Other Therapeutic Methods

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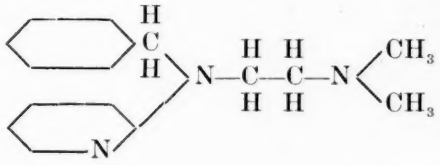
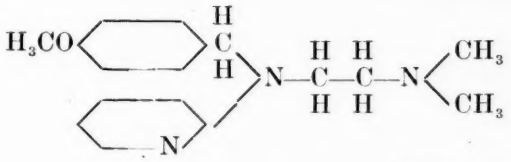
THE purpose of this paper is to compare the effectiveness of two antihistaminic drugs, Neoantergan[1-4] and Pyribenzamine[5-7] with other forms of therapy of seasonal allergic rhinitis. When their structures are compared, it is obvious that Neoantergan and Pyribenzamine are chemically identical except for the addition of a methoxy group on the ortho position of the benzyl ring of the former.



N′pyridil-N′benzyl-N-dimethyl ethylenediamine

Pyribenzamine



N′p-methoxybenzyl-N′pyridil-N-dimethylethylenediamine

Neoantergan

In protecting the guinea pig against lethal doses of histamine administered parenterally and by aerosol, Neontergan was found by Winter to be more potent than Pyribenzamine.[8] The former, when injected in lethal doses into mice, is tolerated in higher quantities than Pyribenzamine.

In order to compare these drugs clinically and compare their effectiveness in hay fever with that of injection therapy, the following experiment was devised.

EXPERIMENT

During the spring, summer, and fall of 1947, patients suffering from hay fever, treated at this clinic, were divided into six groups. The treatment for each group was arranged according to the following plan.

Group I.—Patients who received hyposensitization therapy only, perennial, preseasonal, or coseasonal. These patients were given supplemental placebo tablets three to four times daily.

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†Neoantergan was supplied by Merck and Co., Inc., Rahway, N. J. Pyribenzamine is manufactured by Ciba Pharmaceutical Products Inc., Summit, N. J.

‡Formerly Captain, Army of the United States.

Group II.—Patients who received adequate hyposensitization therapy according to the manner described for Group I, and to whom were given supplemental treatment with Neoantergan tablets, three or four or more times daily p.r.n. Fifty and 100 mg. tablets were used.

Group III.—Patients who received adequate hyposensitization therapy as described in Group I, and to whom were given supplemental tablets of Pyribenzamine, three or four or more times daily p.r.n. Fifty milligram tablets were used.

Group IV.—Patients who received injections of sterile saline solution in place of hyposensitization therapy, and were treated with Neoantergan tablets, three or four or more times daily p.r.n. Fifty and 100 mg. tablets were used.

Group V.—Patients who received injections of sterile saline instead of hyposensitization therapy and were given Pyribenzamine tablets three or four or more times daily p.r.n. Fifty milligram tablets were used.

Group VI.—Patients who received injections of sterile saline instead of hyposensitization therapy, and to whom placebo tablets were given in place of antihistamine medication.

One hundred forty-seven patients, 63 per cent males and 37 per cent females, whose ages ranged from 16 to 40 years, were included in the project. Patients were seen several times a week, and gave an accurate day-by-day description of symptoms, number of tablets taken, and side reactions experienced. Placebo tablets were of sugar and made to look exactly like the Neoantergan. Those patients receiving coseasonal therapy were seen daily. Complete blood counts were performed every ten days on patients receiving histamine antagonists. Local daily pollen counts were made and recorded. The results were evaluated according to the patients' own daily description, the observer's objective findings, and an over-all impression by the patient at the termination of the season. Patients who experienced 50 per cent or greater relief of symptoms were considered benefited by the therapy. These individuals were further classified into three groups, according to the extent of relief obtained: 50 per cent, 75 per cent, and 100 per cent alleviation of symptoms. Patients considered 100 per cent relieved had no symptoms at all. Those classified as 75 per cent relieved had very mild symptoms, which, though present, caused little discomfort. Those considered 50 per cent relieved had occasional symptoms which were noticeable but caused no severe discomfort. These symptoms were at least one-half as intense as symptoms prior to the institution of therapy or experienced in previous years.

RESULTS

Table I indicates the results of the different methods of treatment. Eighty-seven per cent of the patients receiving hyposensitization therapy alone were benefited; 78.9 per cent of the individuals receiving hyposensitization therapy and Neoantergan had satisfactory relief; 89.4 per cent of those receiving hyposensitization injections supplemented by Pyribenzamine had over 50 per cent relief of symptoms; 71.5 per cent of the patients who were treated with

TABLE I

GROUP	TREATMENT	NUMBER OF PATIENTS	PER CENT BENEFITED
I	Hyposensitization Therapy	23	87
II	Hyposensitization Therapy Supplemented by Neoantergan	38	78.9
III	Hyposensitization Therapy Supplemented by Pyribenzamine	26	89.4
IV	Neoantergan	28	71.5
V	Pyribenzamine	27	70.3
VI	Placebo	5	20

TABLE II

GROUP	NUMBER OF PATIENTS	DEGREE OF RELIEF			
		LESS THAN 50 PER CENT	50 PER CENT	75 PER CENT	100 PER CENT
I	23	3 (13 %)	3 (13 %)	9 (39.1%)	8 (34.9%)
II	38	8 (21.1%)	6 (15.8%)	13 (34.1%)	11 (29 %)
III	26	3 (11.5%)	5 (19.2%)	5 (19.2%)	13 (50 %)
IV	28	8 (28.5%)	3 (10.7%)	12 (42.9%)	5 (17.9%)
V	27	8 (29.7%)	4 (14.8%)	12 (44.4%)	3 (11.1%)
VI	5	4 (80 %)	1 (20 %)		

Neoantergan alone were significantly relieved; while 70.3 per cent of those given Pyribenzamine alone were benefited. Twenty per cent of the small group which received only placebos were relieved.

Table II shows the degree of relief experienced by the patients who were benefited by the treatment.

It would appear from these figures that hyposensitization therapy alone was just as effective as hyposensitization supplemented by Pyribenzamine or Neoantergan. Actually, this is not true, and can be clarified by evaluating the results according to the type of hyposensitization therapy employed.

TABLE III

GROUP	TYPE OF HYPOSENSITIZATION	NUMBER OF PATIENTS	DEGREE OF RELIEF			
			LESS THAN 50 PER CENT	50 PER CENT	75 PER CENT	100 PER CENT
I	Preseasonal and Perennial	11			4 (36.4%)	7 (63.6%)
	Coseasonal	12	3 (25 %)	3 (25 %)	5 (41.7%)	1 (8.3%)
II	Preseasonal and Perennial	15		3 (20 %)	4 (26.7%)	8 (43.3%)
	Cosasonal	23	6 (26.1%)	4 (17.4%)	9 (39.1%)	4 (17.4%)
III	Preseasonal and Perennial	8	1 (12.5%)		2 (25 %)	5 (62.5%)
	Coseasonal	18	2 (11.1%)	5 (27.8%)	3 (16.7%)	8 (45.4%)

Table III indicates that of those who received injection therapy alone, 47 per cent had either perennial or preseasonal therapy, and 53 per cent had the coseasonal form. One hundred per cent of the patients who received perennial or preseasonal therapy benefited from the treatment, 63.6 per cent having complete relief, and 34.6 per cent experiencing 75 per cent relief. These results are better than those usually reported with this form of therapy, and since the

group of patients is small the figures cannot be considered reliable. Arbesman[9] reports 67 per cent of his patients improved with preseasonal therapy, but preseasonal and perennial therapy given in this clinic in previous seasons have benefited almost 90 per cent of our patients. Seventy-five per cent of the patients given coseasonal therapy in Group I were relieved. Twenty-five per cent of these experienced 50 per cent alleviation of symptoms, 41.7 per cent had 75 per cent relief and only 8.3 per cent were completely relieved. These figures show clearly that perennial and preseasonal therapy are more effective than coseasonal injection therapy.

In Group II which received hyposensitization therapy supplemented by Neoantergan, 39.5 per cent of the patients had perennial or preseasonal therapy, while 60.5 per cent were treated coseasonally. The only patients in this group who failed to benefit from treatment were in the 60.5 per cent group receiving coseasonal injections. These failures constituted 26.1 per cent of the patients given this form of therapy, and are about the same percentage of failures as in Group I. It would appear from these figures that Neoantergan had little effect in the cases where coseasonal therapy failed.

Optimum results were obtained in Group III where patients received hyposensitization therapy supplemented by Pyribenzamine. Coseasonal therapy was given to 69.2 per cent of the patients, and 88.9 per cent of them were benefited by treatment. All but one patient receiving perennial or preseasonal therapy had excellent relief in this group. The total result of 89.4 per cent response to treatment in Group III has added significance, in view of the fact that here most of the patients received the least effective form of injection therapy.

Almost identical results were obtained from the hay fever sufferers treated with the antihistamine drugs alone. Seventy per cent of the patients taking Pyribenzamine and 71.5 per cent of the patients on Neoantergan had satisfactory relief. These figures are very close to the average of most other investigators from antihistaminic agents alone.[10] In these groups, as far as therapeutic results are concerned, both drugs were equal in value.

Group VI, composed of patients who received only placebos, was devised to demonstrate the effect of the psyche in hay fever. These patients thought they were receiving the same treatment as the others. One patient, 20 per cent of the group, experienced 50 per cent alleviation of symptoms, and definitely stated that the treatment helped. Another patient, although not objectively benefited by treatment, made the following statement, "My nose feels just the same, but for some reason or other I feel better." In considering only five cases, no conclusions can be drawn, but the observations clearly demonstrate the psychosomatic aspect in hay fever.

The dosage of Pyribenzamine in most instances was 50 mg. three or four times daily. However, on several occasions it was found that higher doses were more efficacious, especially when the pollen count was very high. One patient took nine 50 mg. tablets in one day and experienced relief with no increase in side reactions.

Neoantergan was administered in 50 and 100 mg. doses, usually three or four times daily. After using the drug a short time it became apparent that the 100 mg. tablets produced more satisfactory results than the smaller dose. In a few cases when symptoms were more severe, as many as seven 100 mg. tablets were taken for relief. Side reactions were more frequent with the larger dose, but the increased effectiveness was considered important enough to outweigh the toxicity.

Side effects experienced by patients taking the antihistamine drugs are noted and compared in Table IV. Neoantergan produced side reactions in 70 per cent. These in the order of their frequency were: drowsiness, nausea, headache, weakness, dizziness, and diarrhea. These side effects disappeared spontaneously within three days in 61 per cent of those who reacted. In the remaining 39 per cent, symptoms persisted and were very mild in all but two cases. These two patients had to be given another medication because of persistent diarrhea in one and severe drowsiness in the other. Side effects were more pronounced with the 100 mg. dose, and in 11 instances the dosage had to be reduced from 100 mg. to 50 mg. because of their severity. After lowering the dose, side reactions remained in three of the patients, but were not severe.

TABLE IV

MEDICATION	NUMBER OF PATIENTS	NUMBER OF PATIENTS EXPERIENCING SIDE EFFECTS						
		DROWSINESS	NAUSEA	HEADACHE	WEAKNESS	DIZZINESS	DIARRHEA	SWEATING
Placebo	28	5						
Neoantergan	66	34	3	3	3	2	1	
Pyribenzamine	53	16	1	1				1

Pyribenzamine produced side reactions in 36 per cent of the patients, about one-half the incidence encountered with Neoantergan. The reactions in order of frequency were: drowsiness, weakness, nausea, and sweating. They disappeared within two days in all but two cases, and in both cases symptoms were mild.

It is interesting to note that 18 per cent of the patients receiving placebo tablets also complained of drowsiness. This occurred after the patients began to compare symptoms. The drowsiness disappeared within two days in all but one case. That patient had persistent drowsiness, and finally refused to take the placebo. The side-effect disappeared after it was discontinued.

Prolonged use of both Neoantergan and Pyribenzamine produced no effect on the blood picture of those patients taking the drugs. Complete blood counts taken every ten days on these patients showed no significant change.

DISCUSSION

There are several points borne out in this study which indicate that hyposensitization therapy is a more effective and reliable method of treatment of hay fever than antihistamine agents alone. The results in Table I show that hyposensitization therapy, both alone and when supplemented by histamine antagonists, produces greater benefit than antihistamine agents alone. An

aspect which has not been emphasized is the frequent development of seasonal asthma in hay fever sufferers. It is noteworthy that while under observation ten patients developed asthma. Five had received no therapy when the asthmatic symptoms began, two patients were receiving coseasonal therapy and Pyribenzamine, one was being treated coseasonally without supplemental medication, one was on Pyribenzamine alone, and one patient received only Neoantergan. None of the patients who had been given preseasonal or perennial hyposensitization therapy developed asthma while under our care this season. The incidence of asthma in the patients not receiving preseasonal or perennial therapy was 10.3 per cent. This is a very significant and formidable figure which must be considered in choosing a form of treatment. It appears that antihistamine drugs do not prevent progression of hay fever to seasonal asthma, although they may relieve hay fever symptoms.

The pollen counts for 1947 at Fort Dix, New Jersey, are presented in Fig. 1. The standard slide exposure instrument recommended by the National Pollen Survey Committee was used and figures recorded as the number of pollen granules/square centimeter of 24-hour slide. Counts in this area were considerably higher than those in Philadelphia, 35 miles away. The highest count recorded by us in 1947 was 225 granules/square centimeter, on Sept. 2, 1947. This is equivalent to 800 pollen granules/cubic yard of air.[11] In general, the intensity of hay fever in this area was considered moderately severe in 1947.

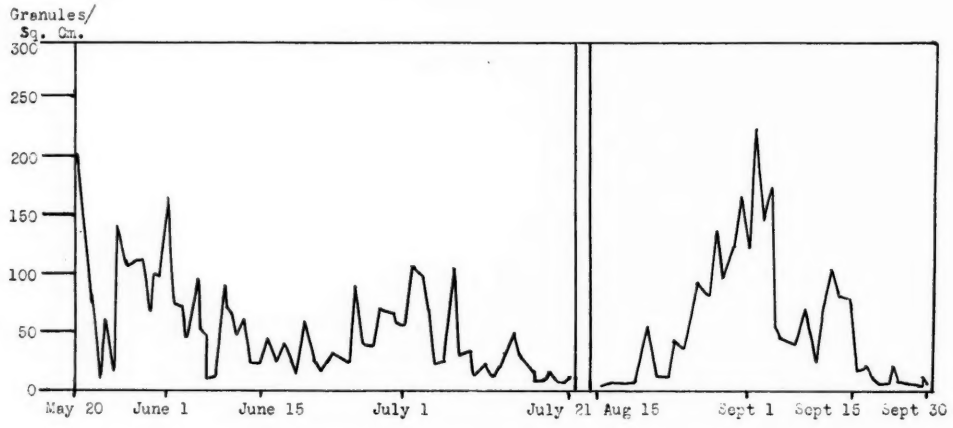

Fig. 1.—Pollen counts, Fort Dix, New Jersey, 1947.

Observations of the response to Neoantergan lead us to conclude that it is another effective histamine antagonist. When used alone in the treatment of hay fever, it was as effective as Pyribenzamine, but when supplementing injection therapy it was less effective. Further observations are necessary to determine whether this difference is actual or merely coincidental. The incidence of side reactions from Neoantergan is greater than from Pyribenzamine, but it should be noted that most side effects clear in a few days. It is interesting that many patients who were not relieved by Pyribenzamine obtained relief from Neoantergan. The reverse of this also occurred. Thus, if relief is not experienced from one histamine antagonist, the use of another may be of value.

As a result of our study we conclude that the treatment of choice for seasonal allergic rhinitis is specific hyposensitization therapy, preseasonal or perennial, supplemented by antihistamine agents. This routine will provide maximum relief and possibly prevent the complication of asthma.

SUMMARY

1. Six different routines of therapy of hay fever are compared for effectiveness.

2. When compared with Pyribenzamine, Neoantergan is found to produce more side reactions and have slightly less effect in supplementing injection therapy.

3. Histamine antagonists do not prevent the development of seasonal asthma.

4. Hyposensitization therapy, preseasonal or perennial, supplemented by antihistamine medication, is the treatment of choice for hay fever.

We are indebted to Dr. Robert A. Cooke for his kind assistance.

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Correspondence

New York, N. Y.
April 9, 1948.

To the Editor:

The report, "Clinical Effects of Epinephrine by Inhalation" (J. Allergy **19:** 129, 1948) by Robert L. Benson and Frank Perlman, is so completely different from my experience during the last eleven years that I read the article critically.

The conclusions of the writers that inhalation of a fine spray of nebulized epinephrine was harmful, even to the point of resulting in mortality five times as great in users as in the nonusers, seemed to me completely without foundation, either from the standpoint of the theoretical background of their argument or from the facts which they presented.

I cannot understand how the authors could include Case 3 in which repeated doses of adrenalin by injection and finally morphine resulted in death.

Excessive use of any medication is manifestly unwise. Having had several thousand patients under my observation, in whom hypodermic administration of adrenalin was abandoned for the use of nebulized epinephrine, I must testify that the ill effects of overdosage of systemic medication with adrenalin are worse than the nebulized spray. Under both circumstances it should be apparent that a seeming requirement for large doses of either hypodermic adrenalin or nebulized epinephrine should be met with by the use of other measures, such as physiologically or allergically directed therapy.

This paper appeared to me to be destructive without any constructive suggestions. The authors may advise against the use of nebulizers, but it would be interesting to know what they advise in their place. It is true that the aerosols of epinephrine and Neosynephrin may be used to excess. This is also true of alcohol. The skillful physician does not seem to me to be performing his function if he treats everybody as if he were a potential addict. In my own clinic and office practice the patient is taught that other measures of treatment are a necessity if frequent use of epinephrine compounds is apparently dictated by recurrence of severe asthma. Both nebulized and injectable epinephrine should be stopped until bronchial relaxation is achieved, as suggested in this journal (J. Allergy **14:** 296, 1943).

In my experience nebulized epinephrine produced by a nebulizer that creates an aerosol of small particle size is one of the most valuable therapeutic agents in the management of bronchial asthma.

Alvan L. Barach, M.D.

Boston, Mass.
April 27, 1948.

To the Editor:

I have just read the report, "Clinical Effects of Epinephrine by Inhalation" by R. L. Benson and F. Perlman. Inasmuch as their experiences are so utterly different from my own and those of other investigators I should like to make the following comments.

I have just reviewed 513 clinical histories of patients with serious bronchial asthma that I have had occasion to manage, or see in consultation during the past ten years. In this series there have been three deaths and these could be directly attributed to the injudicious use of morphine and inadequate bronchiolar evacuation. Only one patient of the series had ever used a bronchodilator spray. On the other hand, the greater majority of the patients had employed bronchodilator aerosols as an adjunct in their management.

The authors discuss a so-called "anatomical pattern of harm" from the excessive bathing of the respiratory mucosa by concentrated epinephrine solutions and then subse-

quently state that they were aware that the changes which were discussed could be found in the classical surveys of the pathology of asthma reported long prior to the use of epinephrine aerosols. I have just reviewed the autopsy data from sixty patients admitted to the Boston City Hospital since 1934 with serious bronchial asthma. These findings show but very slight variation, if any, from the classical autopsy reports as well as those discussed by Benson and Perlman. Evidence of infection of the entire tracheobronchial tree, parenchymal involvement, epithelial metaplasia, subepithelial infiltration with mononuclear cells, *particularly plasma cells,* breaks in the continuity of the mucosa, and widespread evidence of bronchiolar obstruction among other pathology was demonstrated alone or collectively in all our cases. The most significant thing is that only one patient of this entire group (as far as could be determined from the clinical history) had employed any bronchodilator therapy prior to death and he had not employed same for fully two weeks before death. He finally died of coronary insufficiency and congestive heart failure.

A review of our autopsies revealed that the patients received a wide variety of medicaments before death: adrenalin, subcutaneously and intramuscularly, innumerable barbiturates, aminophyllin by various routes, intravenous therapy, digitalis, oxygen, ether, paraldehyde, and morphine. I cannot indict any of these medicaments, with the possible exception of morphine, as contributing to the death of these patients. Furthermore, in reviewing the death reports by other investigators, I could find no reference suggesting any possible indictment of the inhalatory route as a precipitating or contributory event in the death of their patients.

I would conclude from Benson and Perlman's autopsy data (10 cases and 3 case reports) that in no instance have they described a death which could be fully attributed to the inhalation of a properly nebulized spray of epinephrine. On the contrary, an analysis of two of their three reported cases reveals the use of a variety of therapeutic agents. Case 2 had received many medicaments for symptomatic relief. Case 3 had received repeated doses of adrenalin by injection and morphine, in addition to other medications.

There are a number of features in this paper which do have constructive value and should be strongly stressed. It is well appreciated that inhalation of bronchodilator aerosols is of value for the relief of asthmatic paroxysms. There are three well-known bronchodilator agents, namely 1:100 epinephrine, a racemic brand of epinephrine 1:50, and a new Isopropylamino ethanol (Isuprel) 1:200. These should be employed only with the use of a nebulizer capable of delivering aerosols of small particulate size. In this way, one may avoid deposition of large particles with irritation and rapid absorption from the trachea. The patient should be carefully instructed to use the nebulizer only when absolutely necessary and then in dosages recommended by his physician. He should be instructed never to swallow or aspirate during and following the treatment. These instructions are important and do not differ in principle from the careful advice that is the responsibility of the physician when employing any drug in the management of the asthmatic patient. Overdosage and indiscriminate usage on the part of some should not serve as an indictment against the use of a method of therapy which can afford tremendous symptomatic relief safely for a considerable number of asthmatic sufferers. Finally, these patients should be carefully instructed in other methods for obtaining relief of bronchospasm and the principles of bronchial evacuation.

MAURICE S. SEGAL, M.D.

PORTLAND, OREGON
JUNE 2, 1948.

To the Editor:

In answer to the dissenting comments of Dr. Barach and of Dr. Segal we reiterate that our report was based on an impartial survey of 2,236 asthmatics treated over a period of ten years. Of these, 648 admitted the use of epinephrine sprays. Our conclusion that such medication contributed materially to the high mortality was reached through simple mathematical deduction; the death rate was over five times as high in users of the spray than in nonusers.

We emphasize that the traffic in racemic epinephrine for intrabronchial spray originated in the Pacific Northwest about 15 years ago with certain commercial interests. Their agents distributed the product from door to door or sold it over the drug counter without prescription. As a result many thousands of asthmatics throughout this section of the country have treated themselves with the spray, often with relief. When the medication lost its effect, as it usually does, they invariably stepped up the tempo of administration, and it was then that the harmful effects manifested themselves, and they consulted a physician. In many of our cases the damage was already so far advanced that relief was difficult to obtain from any form of medication. Thus far, 51 of these are known to have died. Many times that number including some of the most severe cases have undergone a satisfactory recovery when suitable treatment was substituted for the vasoconstrictor inhalation. Otherwise, since our practice comes mainly from the Northwest, many more fatalities would necessarily have followed.

We have not argued against the right of any physician to prescribe the treatment he thinks may be beneficial, provided he can exercise proper control and prevent abuses. Our objection is mainly to a huge lay distribution of a powerful drug without prescription, and often with disastrous results. In many instances the patient was first seen by us in the hospital in extremis. In a greater number we have not had a chance to direct or modify the dosage until the harm was already done. The problem at present may be localized somewhat in the Northwest. We therefore advise that, before the flood waters of this dangerous traffic reach the Middle West and the Atlantic seaboard, those sections enforce rigorously their laws safeguarding against sale of these powerful drugs without prescription. We personally feel that there are many forms of medication more effective, and certainly safer, than epinephrine by inhalation. The important thing, however, is to prevent this "neighbor to neighbor" promulgation without prescription.

ROBERT L. BENSON
FRANK PERLMAN

American Academy of Allergy

ANNOUNCEMENT

Northwestern University Medical School will offer a 5-day orientation course in allergy under the sponsorship of the American Academy of Allergy, October 25-29 inclusive. The course will comprise a complete and practical coverage of the subject and will utilize teachers not only from Northwestern University, but also from other local and out-of-town medical schools. For particulars direct communications to Department of Allergy, Northwestern University Medical School, Chicago, Ill.

The Committee on Post Graduate Education of the School of Medicine, University of Pittsburgh, announces an Orientation Course in Allergy to be given over a series of ten Thursday afternoons beginning September 2, 1948. The course will include didactic, laboratory and clinical presentations in allergy and all the related specialties. Address all inquiries to Samuel P. Harbison, M.D., Chairman, Committee on Post Graduate Education, Dean's Office, School of Medicine, O'Hara Street, Pittsburgh, Pa.